# Hydration of the Phosphate Group in Double-Helical DNA

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ABSTRACT Water distributions around phosphate groups in 59 B-, A-, and Z-DNA crystal structures were analyzed. It is shown that the waters are concentrated in six hydration sites per phosphate and that the positions and occupancies of these sites are dependent on the conformation and type of nucleotide. The patterns of hydration that are characteristic of the backbone of the three DNA helical types can be attributed in part to the interactions of these hydration sites.

## INTRODUCTION

The nucleotide sequence and solvent composition of nucleic acids affect their thermodynamic behavior, conformation, and interactions. It has been demonstrated by many experiments (Texter, 1978; Chalikian et al., 1994a) that there are differences in the properties of the solvent and counterions at the surface of nucleic acids and in bulk solution. This shell of tightly bound water at the nucleic acid surface is estimated to be about two layers thick or ~4 Å (Chalikian et al., 1994a).

The hydration shell retains its identity at both high and low values of relative humidity (Tao et al., 1987; Lavalle et al., 1990). Various estimates place the number of water molecules per nucleotide to be between 5 and 12. At lower relative humidities, water does not diffuse freely and is located mostly around phosphate groups (Edwards et al., 1984; Milton and Galley, 1986). The fact that water is first removed from the grooves indicates a weaker hydrogen bonding by bases than by phosphates. These observations have been confirmed by neutron quasielastic scattering (Schreiner et al., 1988).

The lower mobility of water at the DNA surface is in accord with the theoretical concept of electrostriction (Rashin, 1993). Charged molecules cause electrostatic contraction (electrostriction) of nearby water molecules and partial loss of their mobility. Release of this partially ordered water upon binding of charged molecules is a source of entropic force (Leikin et al., 1993). The directly measured force between B-DNA molecules (Rau et al., 1984; Rau and Parsegian, 1992a,b) does not obey the predictions of the electrostatic double-layer theory. When the DNA surfaces are separated by 5–15 Å, their intermolecular interaction is only weakly dependent on ionic strength and is independent on DNA molecular size. Therefore, at distances shorter than ~10 Å, interaction between biomolecules is thought to be driven more by hydration forces than by electrostatic or van der Waals forces.

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Crystallographic (Westhof, 1988, 1993; Westhof and Beveridge, 1989; Saenger, 1987; Berman and Schneider, 1998), NMR (Wüthrich, 1993), and theoretical (Clementi, 1983; Smith and Pettitt, 1994; Beveridge and Ravishanker, 1994; Jayaram and Beveridge, 1996) studies have been used to describe the structural features of bound waters. The focus of studies for many years was on base hydration, since the "spine of hydration" was first described to be in the minor groove of the crystal structure of a B-DNA dodecamer (Drew and Dickerson, 1981). NMR methods confirmed the existence of these minor groove bound waters (Liepinsh et al., 1992; Kubinec and Wemmer, 1992), indicating that their lifetimes are sufficiently long to be detected by NMR techniques. A few waters in the major groove of B-DNA have their relaxation times just below the limit for unequivocal localization (Denisov et al., 1997), and hydration, especially of phosphate groups, is very dynamic and is undetectable by NMR methods.

Theoretical methods have also demonstrated the characteristics of the groove-bound water molecules, although recent computational analyses have indicated that for at least a part of the time, these water molecules may be replaced by cations (Young et al., 1997a,b). Alternations of water and sodium cations have recently been observed in the minor groove of a dodecamer crystal structure (Shui et al., 1998). $Na^+$ was previously observed to bind to uracil in the minor groove of 5′-AU-3′ (Seeman et al., 1976). Systematic studies of the bases in all duplex DNA crystal structures have shown that the distribution of the water molecules around bases depends more on the chemistry of the bases than the conformation of the nucleotide residue (Schneider et al., 1993).

A statistical mechanical method (Hummer and Soumpasis, 1994) in which the uniform water distribution in bulk solvent is modified by the external field of a macromolecule has been used (Hummer et al., 1995) to predict water structure around several high-resolution crystal structures. The agreement between experimentally observed and predicted water positions has been very good.

The characteristics of hydration around the phosphates have not been analyzed as thoroughly as base hydration. Early studies of A-DNA crystal structures led to the concept of the "economy of hydration" (Saenger et al., 1986), in

which water molecules bridge neighboring phosphate groups and thus stabilize the A form at lower water activities. High-angle neutron scattering studies (Langan et al., 1992) have revealed networks of water molecules linking the phosphate groups in the major groove of A-DNA.

Several analyses of crystal structures have revealed repeating patterns of water molecules hydrogen bonded to bases, but irregularities in hydration patterns around phosphate groups have led to the conclusion that the water structure of the phosphate backbone is less ordered (Eisenstein and Shakked, 1995; Gessner et al., 1994). In A-RNA, charged phosphate oxygens are a part of the water network lining the deep major groove (Egli et al., 1996). A study of the hydration of B-DNA crystal structures has shown some localization of water around phosphate groups (Umrania et al., 1995).

Early theoretical studies used quantum mechanic calculations to predict that six molecules of water constitute the first hydration shell of a phosphate group (Pullman et al., 1975; Langlet et al., 1979). Each charged oxygen is hydrated separately by three water molecules in a tetrahedral arrangement called a "cone of hydration." Subsequent Monte Carlo computer experiments with hydrated dimethyl phosphate (Clementi, 1983; Alagona et al., 1985) and oligonucleotides (Subramanian et al., 1988) confirmed results of quantum mechanic calculations. Cones of hydration have been experimentally observed around phosphate charged oxygens in crystal structures, as reviewed by Westhof (1993). The cone of hydration has also been reported around phosphate groups in a recent molecular dynamics simulation (Duan et al., 1997), but no ordered phosphate hydration has been reported.

In the work presented here, we have made a systematic analysis of water distributions around the phosphates found in DNA helical crystal structures, using the same method that was originally developed for base hydration (Schneider et al., 1993). The results show the nature of hydration patterns as a function of DNA conformation and nucleotide type.

## METHODS

### Selection of structures

A-, B-, or Z-DNA duplexes without cocrystallized drugs, sugar, and phosphate modifications or base mismatches and with crystallographic resolution better than or equal to 2.0 Å were selected from the Nucleic Acid Database (NDB) (Berman et al., 1992) and are listed in Table 1. When the same sequence was determined twice in the same space group, the crystal structure with better refinement statistics was analyzed.

### Construction of the hydrated building blocks

Water molecules within 3.40 Å of phosphate oxygen atoms, O5′, O3′, O1P, or O2P, were determined using the program Dist (Cohen et al., 1995). Hydrated building blocks were built by superimposing the phosphates with their bonded waters on a phosphate template. Water molecules around the overlapped phosphates constitute a hydrated building block.

Over 20 building blocks were assembled using different criteria for their selection. Phosphate groups were first classified according to DNA conformational type, A, B, or Z, and further classified according to DNA conformation type, BI, BII (Drew et al., 1981; Cruse et al., 1986; Privé et al., 1987; Gorenstein, 1994) and ZI, ZII (Wang et al., 1981). The ranges for the conformation angles used for these classifications were those previously determined (Schneider et al., 1997) by considering the values of torsion angles $\epsilon$ (C4′-C3′-O3′-P) and $\zeta$ (C3′-O3′-P-O5′).

Phosphates were also classified according to the type of nucleotide: guanine, adenine, cytosine, or thymine. By enumerating all of the water distributions around each building block, it was possible to combine those with similar distributions and produce a set of unique building blocks (Table 3). For B-DNA, guanine and adenine residues were pooled into a purine building block, and the cytosine and thymine building blocks were merged into a pyrimidine building block. In the BII conformation, only purines were analyzed, because there are too few pyrimidine residues in this conformational class. In A-DNA, adenine and guanine produce similar water distributions and were therefore combined into one purine building block. Cytosine and thymine nucleotides were treated independently because their water distributions showed large differences, especially around O2P. The thymine building block was not analyzed because its sample size is too small. For Z-DNA, only guanine and cytosine nucleotide building blocks were considered. Cytosine phosphates in ZI and ZII conformations are hydrated differently and were treated as separate groups. The sample size of adenine and thymine building blocks was not sufficient for a reliable analysis.

To minimize the effects of crystal packing on phosphate hydration, phosphates closer than 3.00 Å to any symmetry-related DNA atom were not used for construction of the building blocks. Only 16 of 229 analyzed B-DNA phosphates (7%) are involved in these contacts. Fifteen of them are in the BI conformation, so that virtually all contacts of phosphate groups involved in packing are mediated by the backbone in the BI conformation. One phosphate group in A-DNA and 11 phosphate groups in Z-DNA were excluded from analysis because of close packing contacts.

### Determination of hydration sites

The positions of water oxygens in each building block were Fourier transformed into pseudoelectron densities by using the program X-Plor (Brünger, 1992). The method is described in detail elsewhere (Schneider et al., 1993; Schneider and Berman, 1995) and is similar to the one developed by Murray-Rust and Glusker (1984), who calculate probability densities rather than Fourier transforms of the analyzed points. Peaks in the resulting density maps were manually fitted using the program O (Jones et al., 1991). The positions of these peaks represent sites where water molecules are most likely to be hydrogen bonded to phosphate oxygens and are called hydration sites.

Densities at positions of different hydration sites vary, and not all sites have the same weight for detecting likely positions of localized water molecules. The relative significance of individual hydration sites was estimated according to the following equation:

$$O(i) = S\rho(i)/\Sigma\rho(j)$$

where $O(i)$ is an occupancy of a hydration site $i$, and $\rho(i)$ is its electron density. $\Sigma\rho(j)$ is the sum of densities at all fitted sites of a building block. $S$ is the number of water molecules in a building block divided by the number of phosphate groups contributing to the building block.

For the B-DNA building blocks, the positions of hydration sites were further refined with the program SHELXL (Sheldrick, 1993), using the protocol previously described (Schneider and Berman, 1995). The hydration site positions were refined for the three B-DNA phosphate building blocks. The $R$ factor of the anisotropic refinement was 17% for BI pyrimidines, 22% for BI purines, and 14% for BII purines, and the average temperature displacement factor ($B$ factor) was 47 Å$^2$. These refinement characteristics are similar to those of the refinement of the hydration sites around B-DNA bases (Schneider and Berman, 1995). The average posi-

**TABLE 1 Structures used in the study**

| Code | Sequence | Conformation | Reference |
|---|---|---|---|
| BDJ008 | C C A A G A T T G G | B | Privé et al. (1987) |
| BDJ017 | C C A G G C C T G G | B | Heinemann and Alings (1989) |
| BDJ019 | C C A A C G T T G G | B | Privé et al. (1991) |
| BDJ025 | C G A T C G A T C G | B | Grzeskowiak et al. (1991) |
| BDJ031 | C G A T T A A T C G | B | Quintana et al. (1992) |
| BDJ036 | C G A T A T A T C G | B | Yuan et al. (1992) |
| BDJ051 | C A T G G C C A T G | B | Goodsell et al. (1993) |
| BDJ052 | C C A A G C T T G G | B | Grzeskowiak et al. (1993) |
| BDJ060 | C T C T C G A G A G | B | Goodsell et al. (1995) |
| BDJB27 | C C A G G C C T G G | B | Heinemann and Hahn (1992) |
| BDJB44 | C C A A C I T T G G | B | Lipanov et al. (1993) |
| BDJB48 | C G A T C G A T C G | B | Baikalov et al. (1993) |
| BDJB50 | C C A G G C C T G G | B | Hahn and Heinemann (1993) |
| BDJB57 | C C A G C G C T G G | B | Lipscomb et al. (1995) |
| BDL001 | C G C G A A T T C G C G | B | Drew et al. (1981) |
| ADFB62 | G C G C G C | A | Mooers et al. (1995) |
| ADH008 | G C C C G G G C | A | Heinemann et al. (1987) |
| ADH026 | G G G C G C C C | A | Shakked et al. (1989) |
| ADH029 | G G G C G C C C | A | Shakked et al. (1989) |
| ADH033 | A T G C G C A T | A | Clark et al. (1990) |
| ADH038 | G T G T A C A C | A | Thota et al. (1993) |
| ADH047 | G T G C G C A C | A | Bingman et al. (1992) |
| ADH070 | A C G T A C G T | A | Willcock et al. (1996) |
| ADH078 | C C C T A G G G | A | Tippin and Sundaralingam (1996) |
| ADHB11 | G G U A U A C C | A | Kennard et al. (1986) |
| ADHB91 | G C G C G C G C | A | Mooers et al. (to be published) |
| ADHB92 | G C G C G C G C | A | Mooers et al. (to be published) |
| ADHB94 | G C G C G C G C | A | Mooers et al. (to be published) |
| ADHB10 | G C G C G C G C | A | Mooers et al. (to be published) |
| ADJ049 | C C C G G C C G G G | A | Ramakrishnan and Sundaralingam (1993) |
| ADJ050 | G C G G G C C C G C | A | Ramakrishnan and Sundaralingam (1993) |
| ADJ051 | G C G G G C C C G C | A | Ramakrishnan and Sundaralingam (1993) |
| ADJ065 | A C C G G C C G G T | A | Gao et al. (1995) |
| ADJ067 | A C C G G C C G G T | A | Gao et al. (1995) |
| ADJ075 | G C A C G C G T G C | A | Ban and Sundaralingam (1996) |
| ADJB61 | C C I G G C C C G G | A | Ramakrishnan and Sundaralingam (1995) |
| ADJB88 | G C G C G C G C G C | A | Tippin et al. (1997) |
| ZDF001 | C G C G C G | Z | Wang et al. (1979) |
| ZDF002 | C G C G C G | Z | Gessner et al. (1989) |
| ZDF028 | C G C G C G | Z | Kagawa et al. (1991) |
| ZDF029 | C G C G C G | Z | Egli et al. (1991) |
| ZDF035 | C G C G C G | Z | Bancroft et al. (1994) |
| ZDF039 | C A C G C G / C G C G T G | Z | Sadasivan and Gautham (1995) |
| ZDF052 | C G C G C G | Z | Ohishi et al. (1996) |
| ZDF060 | T G C G C A | Z | Harper et al. (1998) |
| ZDFB04 | C G C G C G | Z | Chevrier et al. (1986) |
| ZDFB05 | C G C G C G | Z | Chevrier et al. (1986) |
| ZDFB10 | C G U A C G | Z | Geierstanger et al. (1991) |
| ZDFB11 | C A C G T G | Z | Coll et al. (1986) |
| ZDFB12 | C G C G U G | Z | Coll et al. (1989) |
| ZDFB21 | C G C G C G | Z | Ginell et al. (1990) |
| ZDFB24 | C G U A C G | Z | Zhou and Ho (1990) |
| ZDFB25 | C G C G C G | Z | Van Meervelt et al. (1990) |
| ZDFB31 | C G U A C G | Z | Schneider et al. (1992) |
| ZDFB36 | C G C G C G | Z | Cervi et al. (1993) |
| ZDFB41 | C G T A C G | Z | Parkinson et al. (1995) |
| ZDFB42 | C G T A C G | Z | Parkinson et al. (1995) |
| ZDFB43 | C G C G C G | Z | Moore et al. (1995) |
| ZDFB51 | C G C G C G | Z | Peterson et al. (1996) |

tional shift of the sites during the refinement was 0.3 Å, so that manual fitting can be considered reliable.

### Hydration of phosphates in dinucleotides

Phosphate hydration was modeled for dinucleotide steps in B-, A-, and Z-DNA conformations. Dinucleotide templates were constructed using the average geometries derived from higher resolution DNA structures (Schneider et al., 1997). The hydrated building blocks corresponding to the particular conformation were overlapped onto these templates. Densities of water distributions in the diphosphates were calculated using Fourier-averaging techniques, and the hydration sites were fit as described above.

## RESULTS

The results are summarized in Tables 2–4 and Figs. 1–3. Extended versions of the tables and water distributions of all studied building blocks can be found on an NDB web site (http://ndbserver.rutgers.edu/NDB/archives/).

### Extent of hydration

Table 2 summarizes the average extent of hydration of crystal structures of oligonucleotides in the B, A, and Z conformational classes. Most of these water molecules are hydrogen bonded directly to DNA atoms and are therefore in the first hydration shell. The average number of crystallographically ordered water molecules per nucleotide in these structures is between four and five. This represents about half of the total stoichiometric "water of hydration" estimated from thermodynamic, spectroscopic, and other experiments (Falk et al., 1962, 1970; Chalikian et al., 1994b). Ordered water molecules in crystals of oligonucleotides therefore represent a significant fraction of their water of hydration.

The sum of the number of water molecules located in the first hydration shells of the phosphates and bases closely matches the total number of ordered waters in B- and A-DNA (Table 2). Because there are only a few second shell water molecules and few waters are hydrogen bonded solely to the deoxyribose oxygen O4′, it can be concluded

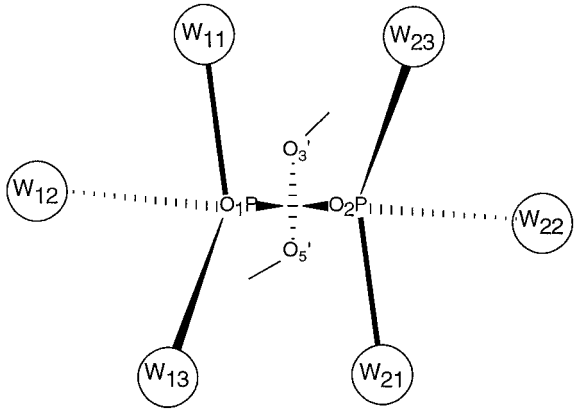


FIGURE 1 A scheme of the first hydration shell around a phosphate group in double-helical DNA.

**TABLE 2 Average hydration of the analyzed structures**

| DNA conformation* | Waters per nucleotide | | |
|---|---|---|---|
| | Total# | Ph§ | Base§ |
| B-DNA | 4.6 | 2.5 | 2.1 |
| A-DNA | 4.2 | 2.2 | 2.1 |
| Z-DNA | 5.3 | 3.6 | 3.2 |

*The average numbers of crystallographically ordered water molecules are listed for each conformational type. The averages were based on 15 B-DNA, 22 A-DNA, and 22 Z-DNA structures listed in Table 1.
#Number of all crystallographically ordered water molecules per nucleotide residue.
§Number of water molecules closer than 3.40 Å from any of the phosphate (Ph) or base (Base) atoms per nucleotide residue. For A- and Z-DNA, only nucleotides containing nonmodified guanine, adenine, cytosine, and thymine were considered.

that hydration shells of phosphate groups and bases overlap weakly in A- and B-DNA. In Z-DNA, the sum of phosphate and base waters (6.8) is larger than the total number of ordered waters (5.3), and the overlap between phosphate and base hydration shells is significant. This is consistent with the existence of a continuous network of hydrogen-bonded water molecules linking phosphates and bases in Z-DNA.

The numbers of ordered waters hydrogen bonded to phosphates is slightly higher than numbers of waters bonded to bases. C-G base pairs have six, and T-A have five potential donors or acceptors of hydrogen bonds; localization of water molecules around the bases is facilitated by more or less concave walls of minor and major grooves. Of the four phosphate oxygens, only two partially charged phosphate oxygens, O1P and O2P, are significantly hydrated. The ester oxygens O5′ and O3′ form only ~15% of all contacts to the phosphate group. Moreover, almost all water contacts to O5′ and O3′ are longer than 3.1 Å, indicating only weak hydrogen bonding of waters primarily bound to the charged oxygens O1P or O2P.

The extent of hydration for the different types of phosphate building blocks is summarized in Table 3. For every sample set analyzed, most of the water molecules contact the charged phosphate oxygens O1P and O2P; only 10–15% of all contacts are with the ester oxygens O3′ and O5′. In most cases, O1P and O2P are hydrated to approximately the same extent. However, in BI pyrimidines O2P is hydrated significantly more than O1P. Z-DNA phosphates are hydrated the most and A-DNA the least, but for each conformational class, the extent of phosphate hydration is the same regardless of nucleotide type.

### Geometry of hydration

Fig. 1 is a schematic of the six sites of hydration that were found in this analysis. The hydration sites for O1P are labeled W11, W12, and W13, and the sites for O2P are labeled W21, W22, and W23. The densities for each building block are shown in Fig. 2. The geometrical features of the hydration sites are listed in Table 4.

**TABLE 3 Statistics of the hydrated building blocks**

| DNA conformation | Nucleotide type | Waters* | Residues# | Waters per residue§ | Water contacts to¶ | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | O1P | O2P | O3′ | O5′ |
| BI | Pyrimidine | 201 | 85 | 2.36 | 35 | 50 | 5 | 10 |
| BI | Purine | 145 | 60 | 2.42 | 45 | 45 | 5 | 5 |
| BII | Purine | 151 | 53 | 2.85 | 50 | 40 | 5 | 5 |
| BII | Pyrimidine | 5 | 4 | — | — | — | — | — |
| A | Purine | 209 | 100 | 2.09 | 40 | 50 | 10 | 5 |
| A | Cytosine | 201 | 91 | 2.21 | 40 | 45 | 10 | 5 |
| A | Thymine | 31 | 12 | 2.58 | — | — | — | — |
| Z | Guanine | 345 | 96 | 3.59 | 45 | 40 | 10 | 0 |
| ZI | Cytosine | 129 | 32 | 4.03 | 40 | 45 | 10 | 5 |
| ZII | Cytosine | 29 | 9 | 3.22 | 40 | 50 | 10 | 0 |
| Z | Thymine + adenine | 33 | 12 | 2.75 | — | — | — | — |

*Number of water molecules within 3.40 Å of phosphate oxygens.
#Number of phosphate groups in nucleotides.
§Waters per residue gives the stoichiometric number of water molecules in a building block and indicates the extent of its hydration.
¶Percentage of contacts of water molecules of a building block with the oxygen atoms of a phosphate group.

In every building block analyzed, the water molecules are ~2.8 Å from O1P or O2P; the hydrogen bonding angle W···OP═P is ~125°. In most building blocks, each charged oxygen is hydrated by three hydration sites. These sites occupy the vertices of trigonal pyramids arising from the charged oxygens. Water density is not observed along the P═O1P and P═O2P vectors. Hydration shells of O1P and O2P are connected only weakly, as there is no density observed in the plane defined by the O5′-P-O3′ atoms.

The sites around charged oxygens O1P and O2P are related by an approximately twofold symmetry defined by the intersection of the O1P═P═O2P and O3′-P-O5′ planes. Thus torsion angles W1X···O1P═P═$O_2$P and W2X···O2P═P═O1P of the same building block have similar values. Sites W11 and W21 lie in a broad region with torsion values between 10° and 110°, with an average of 45°; sites W12 and W22 are in a region between 110° and 210°, with an average of 160°. The positions of sites W13 and W23 have the least variable torsion angles, with values close to −80° (280°).

The existence of three tetrahedrally arranged water molecules in the first hydration shell of each phosphate charged oxygen confirms earlier quantum chemistry (Pullman et al., 1975; Langlet et al., 1979) and Monte Carlo simulation studies (Clementi, 1983; Alagona et al., 1985).

## B-DNA phosphate hydration

The hydration of BI pyrimidine phosphates is concentrated in six well-defined regions (Fig. 2 *A*). The hydration site W22, bonded to O2P, has a very high water density and interacts with O5′ and the atom C6 at the pyrimidine edge. All other sites are much weaker but are well separated and clear in density. Densities of both purine building blocks (Fig. 2, *B* and *C*) are more evenly distributed between O1P and O2P. The hydration around O2P is more dispersed, with long bands of density encircling O2P, and the O1P hydration sites have consequently higher occupancies than the O2P sites. In the BII purines, the sites W22 and W23 split into two subsites.

Distributions around BI and BII purine phosphates are similar despite the fact that their conformations are very different. This supports the view that solvent distributions are determined locally by the chemical nature of interacting groups and are only modified by other, more distant neighbors.

## A-DNA phosphate hydration

In A-DNA, the distributions and hydration site positions are similar for purine and cytosine building blocks (Fig. 2, *D* and *E*). The hydration around O1P has a well-defined site, W13, and a split site, W12. The third site, W11, is present only in cytosine phosphates. The sites W21 and W22 at the phosphate oxygen O2P have high densities and are well resolved. Their high occupancies can be attributed at least partially to the interactions between water and close edges of bases in the deep major groove of A-DNA.

In right-handed DNA forms, B and A, the interactions between hydration sites and the neighboring bases and ribose rings are similar. The site W22 is close (~3.5 Å) to the ester oxygen O5′ and to a base atom C8/C6 in all but the A-DNA purine building block. In this case, the W21 site is positioned between O5′ and C8. Similarly, the site W12 interacts weakly with O3′ in all but the BII purine building block.

Fig. 2, *A–E*, illustrates that the structure of phosphate hydration in A-DNA is simpler than in B-DNA. A-DNA distributions are more compact and their sites are clearly defined. The A-DNA hydration sites around O1P are related to B-DNA by a 20° rotation; the sites around O2P are related by a 40°-50° rotation. Positions of the sites hydrogen bonded to O1P depend less on conformation than those of O2P. This is probably because hydration around O2P, which points into the major groove, is systematically influenced by a neighboring base, whereas O1P points into a more randomized environment of symmetry-related molecules.

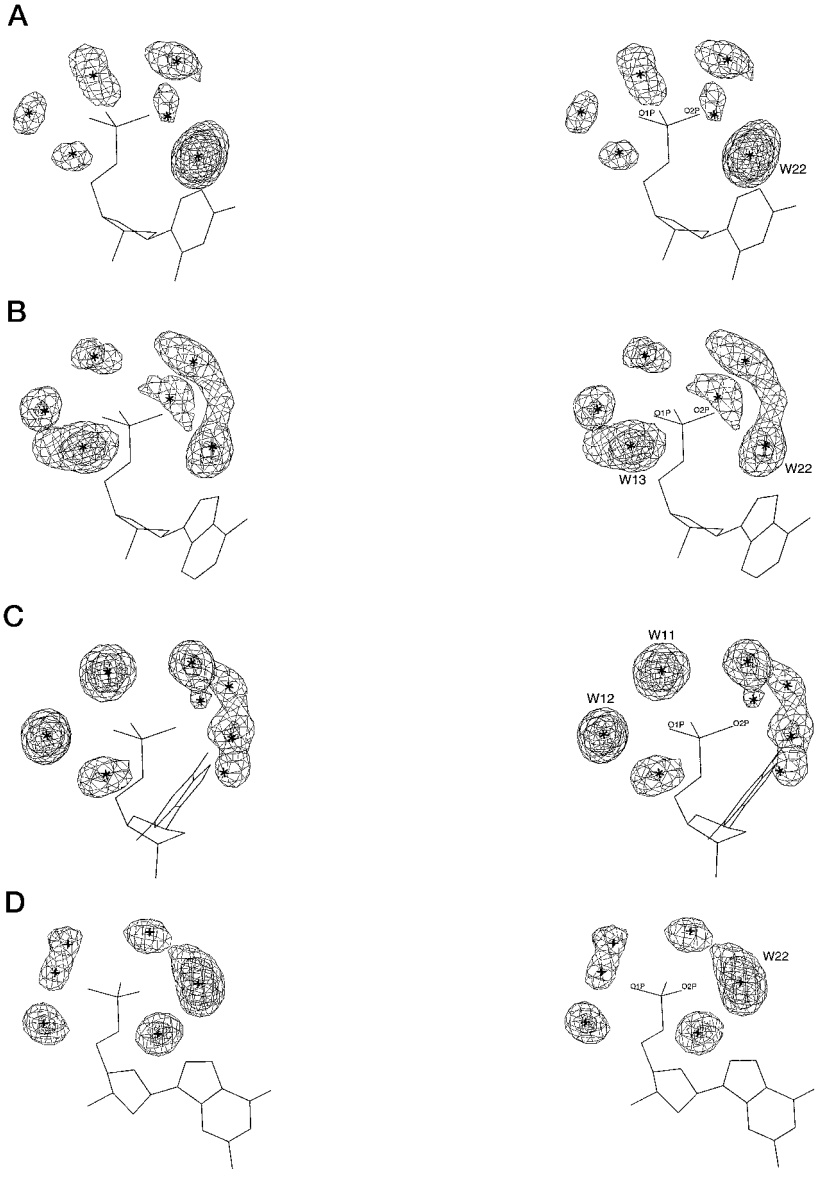


FIGURE 2 Stereo views of water distributions around a phosphate group in double-helical DNA. Crosses indicate positions of hydration sites. The charged phosphate oxygens O1P and O2P and the ester oxygen O3′ are labeled. (*A*) Pyrimidine phosphates in the BI conformation. (*B*) Purine phosphates in the BI conformation. (*C*) Purine phosphates in the BII conformation. (*D*) Purine phosphates in A-DNA. (*E*) Cytosine phosphates in A-DNA. (*F*) Guanine phosphates in Z-DNA. (*G*) Cytosine phosphates in the ZI conformation.

## Z-DNA phosphate hydration

In Z-DNA, the guanine (Fig. 2 *F*) and cytosine (Fig. 2 *G*) phosphate building blocks have very different water distributions, which is consistent with the different conformations for these residues. The guanine phosphates have six clearly separated hydration sites. The ZI cytosine distribution is dominated by the hydration site W22, which has a high density. The hydration around O1P is less defined. The relative rotation between the corresponding guanine and ZI cytosine sites is between 20° and 50°. The Z-DNA hydration sites make no contacts with base atoms and only a few contacts with ribose atoms. In the guanine block, the site W22 contacts O3′, and in the cytosine building block, two ribose carbon atoms are contacted by the sites W22 and W12.

Water distributions are more concentrated in Z-DNA than in B- or A-DNA; this difference is most noticeable for the Z-DNA guanine phosphates. The Z-DNA water distributions are well defined because the sample of Z-DNA crystal structures is very homogeneous.

## Water distributions in dinucleotide steps

The hydration shells of neighboring phosphate groups in a polynucleotide chain can overlap and influence each other. This overlap depends on the distances between the heavily hydrated charged oxygens O1P and O2P and on their mutual orientation. When the distances between the charged oxygens of the following phosphates drop below a distance of ~5.7 Å, water distributions from the neighboring phosphates can overlap. When the distance is larger, the hydration sites from consecutive phosphates may or may not form hydrogen bonds with each other, depending on a particular water distribution around both phosphates and their mutual orientation.

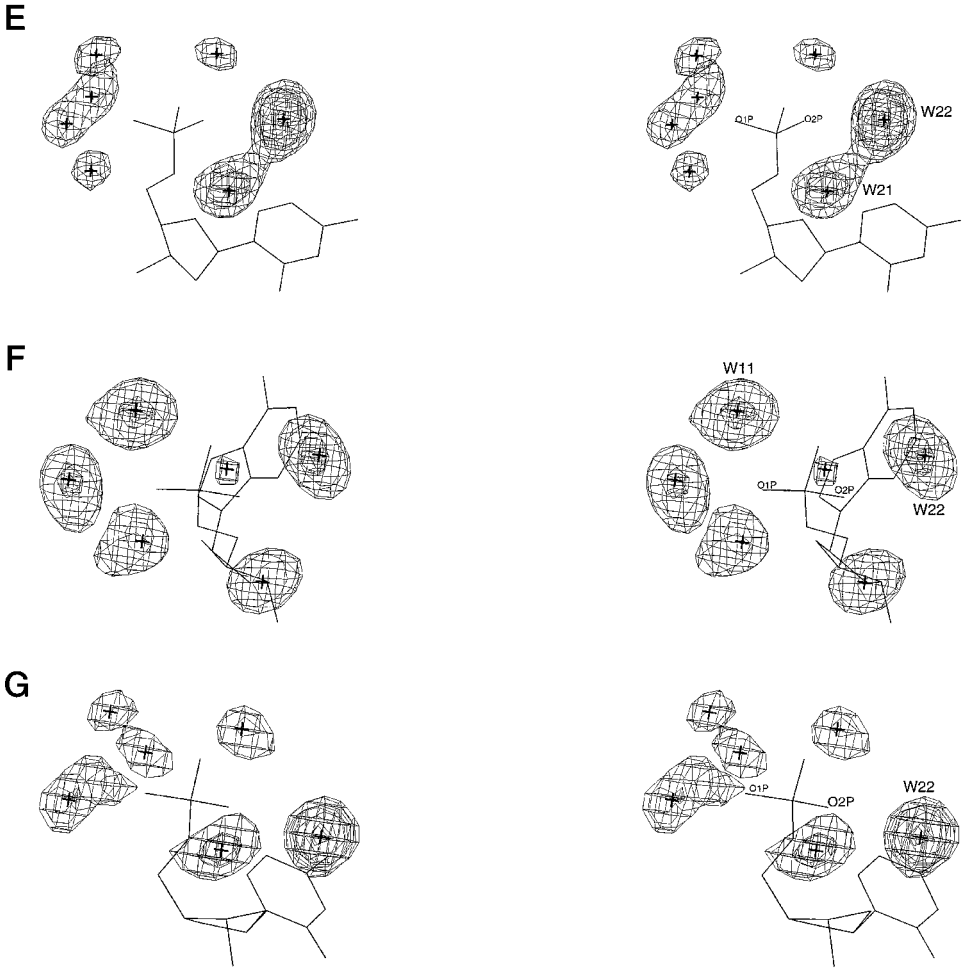


FIGURE 2 Continued.

In B-DNA dinucleotides, hydration spheres of two consecutive phosphate groups interact only marginally, and there are very few or no contacts between their hydration sites. The shortest distance between the charged oxygens is in the BI-BII steps, where the O1P-O1P distance is ~6.2 Å and the densities originally producing the site $W13_i$ of $O1P_i$ from the 5′ phosphate and the site $W11_{i+1}$ of $O1P_{i+1}$ from the O3′ phosphate are partially merged. In all other B-DNA steps, no interactions closer than 3.5 Å were observed between hydration sites from the consecutive phosphates. Fig. 3 *A* shows an example of water distribution around B-DNA phosphates for the BI pyrimidine–BI purine step.

In A-DNA, the average distance between phosphate oxygens O2P is much less than in B-DNA, 5.5 Å. Thus the hydration shells of the consecutive phosphate groups overlap, as illustrated in Fig. 3 *B*. The hydration sites $W21_i$ and $W22_{i+1}$ from individual building blocks overlap and form one strong site located equidistant from the two O2P atoms. Furthermore, the sites $W13_i$ and $W23_{i+1}$ merge into one cloud of density. These water bridges between charged oxygens of two neighboring phosphate groups may help to stabilize a backbone curvature of the A-DNA conformation.

Observation of weak or no overlap between water distributions of neighboring phosphates in B-DNA and their extensive overlap in A-DNA confirms an earlier suggestion that each phosphate group in B-DNA has its own hydration shell, whereas hydration is partially shared by the following phosphates in A-DNA (Saenger et al., 1986). Water molecules bridging two O2P atoms are frequently observed in A-DNA crystal structures (Wahl and Sundaralingam, 1997), and high electron density has been localized in this region by neutron scattering (Langan et al., 1992).

In Z-DNA, the pyrimidine-purine and purine-pyrimidine dinucleotide steps have different conformations, and their hydration patterns are different. In the guanine-ZI cytosine step (Fig. 3 *C*), the two guanine hydration sites $W22_i$ and $W21_i$ form hydrogen bonds with the cytosine sites $W22_{i+1}$ and $W11_{i+1}$. The high-density cytosine site $W22_{i+1}$ also forms a hydrogen bond with the guanine minor groove atom N2. Despite a very short distance (4.8 Å) between the two O2P atoms, the hydration shells of these neighboring phosphates do not overlap as in A-DNA. Rather they interlock in a network of hydrogen bonds linking the charged oxygens and a base atom.

In the ZI cytosine-guanine dinucleotide, the $O1P_{i+1}$ atom of the 3′ guanine residue is close to the ZI cytosine phosphate oxygens $O2P_i$ (5.1 Å) and $O1P_i$ (5.8 Å), but again, the hydration shells of the two phosphate groups interact only

**TABLE 4 Geometry of hydration sites around phosphate groups in DNA**

| Hydration site* | Building block# | Occupancy§ | Torsion¶ | Angle¶ | Distance¶ |
|---|---|---|---|---|---|
| W11 | BI Py | 0.30 | 10 | 125 | 2.6 |
| | BI Pu | 0.25 | 50 | 115 | 2.6 |
| | BII Pu | 0.55 | 30 | 125 | 2.8 |
| | A Cyt | 0.20 | 50 | 140 | 2.9 |
| | Z Gua | 0.80 | 70 | 115 | 3.0 |
| | ZI Cyt a) | 0.35 | 30 | 115 | 2.9 |
| | ZI Cyt b) | 0.35 | 90 | 125 | 2.8 |
| W12 | BI Py | 0.25 | 150 | 115 | 2.8 |
| | BI Pu | 0.40 | 150 | 125 | 2.6 |
| | BII Pu | 0.60 | 170 | 135 | 2.7 |
| | A Pu a) | 0.25 | 110 | 115 | 2.8 |
| | A Pu b) | 0.15 | 150 | 115 | 2.8 |
| | A Cyt a) | 0.25 | 170 | 125 | 2.9 |
| | A Cyt b) | 0.25 | 140 | 110 | 2.7 |
| | Z Gua | 0.75 | 180 | 150 | 2.7 |
| | ZI Cyt | 0.60 | −160 | 130 | 2.7 |
| W13 | BI Py | 0.25 | −90 | 130 | 2.7 |
| | BI Pu | 0.60 | −90 | 130 | 2.7 |
| | BII Pu | 0.30 | −90 | 110 | 2.8 |
| | A Pu | 0.35 | −90 | 130 | 2.9 |
| | A Cyt | 0.20 | −90 | 120 | 3.0 |
| | Z Gua | 0.45 | −30 | 115 | 2.9 |
| W21 | BI Py | 0.25 | 40 | 120 | 2.6 |
| | BI Pu | 0.25 | 20 | 115 | 2.8 |
| | BII Pu | 0.15 | 10 | 130 | 2.7 |
| | A Pu | 0.35 | 100 | 120 | 2.7 |
| | A Cyt | 0.45 | 110 | 105 | 3.0 |
| | Z Gua | 0.55 | 80 | 120 | 2.9 |
| | ZI Cyt | 0.55 | 30 | 120 | 2.7 |
| W22 | BI Py | 0.95 | 140 | 115 | 2.7 |
| | BI Pu | 0.40 | 150 | 120 | 2.6 |
| | BII Pu a) | 0.25 | 120 | 120 | 2.7 |
| | BII Pu b) | 0.35 | 180 | 125 | 2.7 |
| | A Pu | 0.70 | −160 | 135 | 2.8 |
| | A Cyt | 0.65 | −170 | 140 | 2.9 |
| | Z Gua | 0.80 | −150 | 120 | 2.9 |
| | ZI Cyt | 1.00 | 170 | 140 | 2.9 |
| W23 | BI Py | 0.35 | −70 | 135 | 2.6 |
| | BI Pu | 0.30 | −70 | 140 | 2.6 |
| | BII Pu a) | 0.40 | −50 | 125 | 2.8 |
| | BII Pu b) | 0.25 | −120 | 140 | 2.9 |
| | A Pu | 0.30 | −60 | 120 | 3.1 |
| | A Cyt | 0.20 | −50 | 135 | 2.8 |
| | Z Gua | 0.25 | −30 | 115 | 3.0 |
| | ZI Cyt | 0.40 | −60 | 110 | 3.1 |

*The name of a hydration site. Hydration sites labeled W1x (W2x) bind to the charged oxygen O1P (O2P) (Fig. 1).

#DNA conformation and nucleotide type for which a hydration site was determined. Listed are only hydration sites for building blocks with significant numbers of water molecules.

§Occupancies of sites estimated from a formula, $O(i) = \mathbf{S}\rho(i)/\Sigma\rho(j)$, where $\rho(i)$ is electron density at the $i$th site, $\Sigma\rho(j)$ the sum of densities at all fitted sites of a building block. **S** is the number of stoichiometric water molecules in the building block, which equals the number of water molecules divided by the number of residues and is listed in Table 3 under the column Waters per residue.

¶The stereochemistry of hydration sites. For the sites labeled W1x, torsions are defined as W1x · · · O1P = P = O2P, angles W1x · · · O1P=P=O2P, angles W1x · · · O1P=P, and distances W1x · · · O1P. For the sites W2x torsions are W2x · · · O1P = P = O2P, angles W2x · · · O1P = P, and distances W2x · · · O1P. Angles are in degrees, distances in Å.

weakly by merging the cytosine site $W21_i$ and the guanine site $W12_{i+1}$ into one site bridging $O2P_i$ and $O1P_{i+1}$.

## DISCUSSION

Our analysis shows that each charged oxygen in the phosphate group of DNA duplexes has three hydration sites in its first hydration sphere, and both ester oxygens are hydrated only marginally. Distributions are primarily determined by the water-phosphate interactions and then modified according to the particular DNA conformation and base type.

Water has the highest affinity for the phosphate charged oxygens, followed by base hydrophilic oxo groups at both purine and pyrimidine bases, and then both exo- and endo-

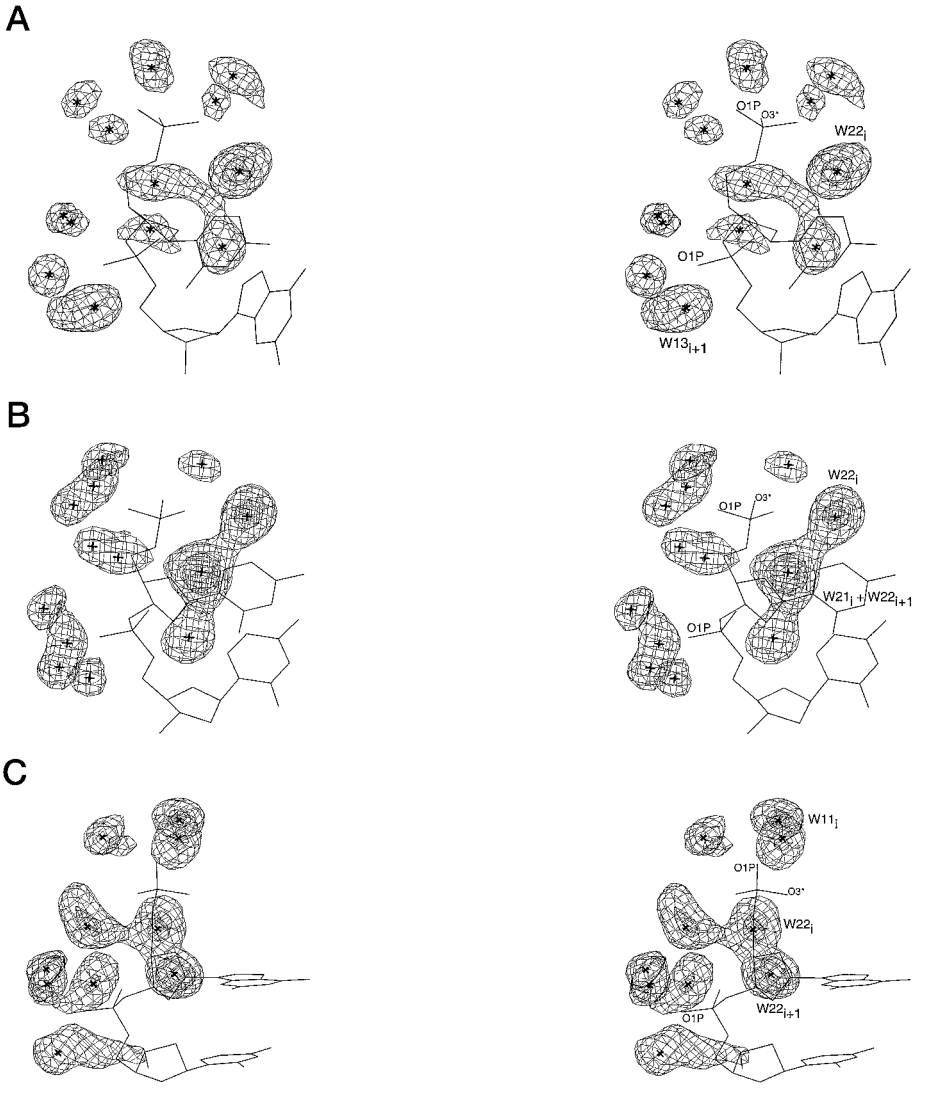


FIGURE 3 Stereo views of water distributions around the phosphate groups of B-, A-, and Z-DNA dinucleotides. Crosses indicate positions of hydration sites. The charged phosphate oxygens O2P and the ester oxygen O3′ at the 5′ end of the chain are labeled. (*A*) BI pyrimidine-BI purine. (*B*) Cytosine-purine in A-DNA. (*C*) Guanine-ZI cytosine in Z-DNA.

cyclic nitrogens of the bases. The deoxyribose ether oxygen O4′ in B- and A-DNA usually shares water with the minor-groove hydrophilic base atom from a previous residue, and it is poorly accessible in Z-DNA, so that it does not constitute an independent unit of DNA hydration. Perhaps surprisingly, the ester oxygens O5′ and O3′ are hydrated least in all three DNA helical forms. O5′ in the right-handed forms is sterically inaccessible; the reasons for poor hydration of O3′ are not clear.

Each DNA conformational type shows a particular pattern of hydration of consecutive phosphate groups (Fig. 3). In B-DNA, first hydration shells of distant phosphate groups can only be linked by second-shell water molecules. Short distances between charged oxygens O2P in A-DNA cause sharing of their hydration shells (Saenger et al., 1986; Wahl and Sundaralingam, 1997). In contrast to A-DNA, hydration of Z-DNA dinucleotides shows that overlap between the water distributions of even very close phosphate groups can result in a network of hydrogen-bonded water molecules.

The bridges formed by the predicted hydration sites between phosphate atoms and neighboring DNA groups correspond closely to the water bridges observed in crystal structures of double-helical DNA. In B-DNA, phosphates are most frequently bridged via their O2P atoms to major-groove base atoms of the same nucleotide. These water bridges correspond to the hydration site W22 in our hydration model. In pyrimidines, most bridges link O2P to the base atom C6. The large frequency of these bridges corresponds to an exceptionally high density of the hydration site W22 in the pyrimidine building block (Fig. 2 *A*). Bridges to the purine atom C8 are similar but slightly less frequent. Contacts to the thymine methyl group are not typical for hydration patterns in B-DNA, because only one-quarter of all O2P···W···thymine bridges contact the thymine methyl group. A similar frequency of contacts was observed for a bridge between O2P and a thymine methyl group of the following residue. These interactions between water and hydrophobic carbon atoms are longer than 3.2 Å, usually close to 3.6 Å. Despite being energetically weak, they are

important for localization of water molecules between the phosphate O2P and a base by restricting the mobility of water molecules. In full analogy, the A-DNA W21 sites are focused by the neighboring base and the site W22 in the ZI cytosine building block by the close deoxyribose.

Two B-DNA phosphate groups are never bridged by a single water molecule. This discrete behavior of water distributions around the individual phosphate groups contrasts with the behavior of the hydration around bases. Relatively small changes in base morphology can change a single row of water molecules in the spine of hydration in a narrow minor groove (Drew et al., 1981) into two parallel rows of waters lining up on the opposite walls of a much wider minor groove (Privé et al., 1991). On the other hand, only substantial changes in the backbone geometry would lead to an overlap between hydration shells of two consecutive phosphates in B-DNA.

The deep and narrow major groove of A-DNA, combined with close distances between phosphate groups, gives rise to more extensive water networks than in B-DNA. The most common bridge is $O2P_i\cdots W\cdots O2P_{i+1}$, where the water molecule hydrogen bonds to O2P atoms from two consecutive phosphate groups. These bridges have also been observed experimentally in neutron scattering experiments (Langan et al., 1992) and are correctly reproduced by our model of phosphate hydration (Fig. 3 *B*). This sharing of water molecules between neighboring phosphate groups in A-DNA has been predicted (Saenger et al., 1986) to contribute to B-to-A conformational transition by a larger economy of A-DNA hydration at lower relative humidities.

A-DNA water molecules hydrogen bonded to O2P make several other types of long contacts to base atoms that are more variable but less frequent than in B-DNA. Two phosphate-to-pyrimidine bridges, $O2P_i\cdots W\cdots C6_i$ and $O2P_i\cdots W\cdots C6_{i-1}$, are present in crystal structures with a similar frequency. Contacts to purines are slightly more populated; the most common connectivity is $O2P_i\cdots W\cdots C8_i$, but $O2P_i\cdots W\cdots C8_{i\pm1}$ is also observed. These contacts to bases are properly modeled by the hydration site W21 of the A-DNA building blocks.

Our observations of water distributions derived from crystal structures can be related to measurements of bulk properties of DNA solutions. Full occupation of all phosphate hydration sites accounts for six water molecules. Pyrimidine bases have at least two and purine bases three localized hydration sites (Schneider and Berman, 1995), yielding two to three water molecules per base. The fully occupied first hydration shell of a nucleotide, therefore, represents between eight and nine water molecules. It accounts for a significant part of the 5–12 tightly bound water molecules estimated independently by thermodynamic and spectroscopic methods (Chalikian et al., 1994a). The unique properties of the water tightly bound to the DNA surface therefore reside mainly in its first hydration shell, which consists of partially ordered water molecules.

The ordered first hydration shell helps to explain the lower mobility of the water close to the DNA surface (Edwards et al., 1984; Milton and Galley, 1986; Schreiner et al., 1988) and the fact that anisotropic reorientation of water molecules around DNA at low humidity (Schreiner et al., 1991) is three to five orders of magnitude slower than in bulk water. Hydration by localized water is also in accord with an interpretation of the strong DNA-water interactions observed in microwave spectra (Edwards et al., 1984). van Zandt (1987) describes this observation through a model in which blocks of water with limited mobility are bound to the DNA surface. An important feature of this model is that water binding is not due to a larger strength of the DNA-water interactions compared to water-water interactions, but to their larger anisotropy.

It has been proposed (Leikin et al., 1993) that the cooperative influence of especially polyvalent cations and water is the source of the hydration force (Marcelja and Radic, 1976), which arises from the work of removing water organized at macromolecular surfaces. Cations bound to DNA reconfigure the water at discrete sites complementary to unabsorbed sites between macromolecular surfaces and create these attractive long-range forces. The hydration force is detected at intermolecular distances between 5 and 15 Å (Rau et al., 1984; Rau and Parsegian, 1992a,b). At a distance of 10 Å, the first hydration shells of both biomolecules are only ~3–4 Å apart, and only one more layer of water can be placed between them. Bringing the two biomolecules closer would indeed result in release of some interfacial water molecules.

The concept of self-organization of the solvation shell at close intermolecular distances is supported by our analyses of water distributions around DNA, which show that a hydrogen-bonded water molecule is more immobilized at places where its movement is restricted by another nearby group. By a simple analogy, we assume that as two macromolecules approach each other, water molecules in their hydration shells become more localized.

The release of water from the significantly ordered first hydration shells into bulk would favorably contribute to the free energy change of the intermolecular interaction by increasing the entropy of the system. Other components of the interaction free energy, such as charge complementarity or steric repulsion, obviously also influence the equilibrium, so that the final coordination of the interacting molecules may be a nonspecific interaction at a distance range of 10 Å or a close specific contact with all or a part of the interfacial water removed.

The proposed significant role of cations in organizing the hydration shell (Leikin et al., 1993) cannot be completely tested by analyses of crystal structures. Because the crystallographic resolution of oligonucleotide structures is not always sufficient for discriminating between water and light ions like $Na^+$ or $Mg^{2+}$, some of the ordered particles traditionally labeled as water may be metal cations. Relatively few metal cations have been observed in ordered positions around phosphates in oligonucleotide crystals, also because they may prefer to bind to phosphates via water molecules and form so-called outer sphere complexes

(Buckin et al., 1994, 1996). Cations are then hard to localize in the highly mobile second solvation shell.

In contrast, metal cations and water are well discriminated in crystal structures of small organic phosphates. Based on these structures, distributions of several metals around phosphates have been determined (Schneider and Kabelác, 1998). The metal ion distributions have the same basic characteristics as water distributions discussed here; they are concentrated in well-defined regions, no cation density is observed along the OP═P bond, and very low or no density is observed in the O5′-P-O3′ plane.

The main conclusion from this and previous studies on DNA hydration is that both bases and phosphate groups have significantly organized hydration shells. The extent of hydration is larger around phosphates, but water is more organized around bases. The phosphate and base building blocks, which were assembled from many crystal structures, can be used to predict a complete hydration shell of any DNA sequence. The sites of high water densities may represent preferential binding sites for hydrophilic residues and can be used for studying DNA interactions with drugs and proteins.

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