A Theoretical Study of a Z-DNA Crystal: Structure of Counterions, Water and DNA Molecules

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To study the effect of solvents and counterions in Z-DNA crystal of *d*(5BrC-G-5BrC-G-5BrC-G), we performed the local energy analysis and then molecular dynamics simulations. Since counterions raise serious caging problems in crystal simulations, it is very important to search for the possible positions before simulations. For this purpose, the local energy analysis was done for the whole crystal volume. It is shown from our simulation that counterions alongwith water molecules play a bridging role to bind adjacent oligomers so as to form the crystal. In this crystal, each water molecule bound to Gua-N2H, either directly or indirectly, hydrates the adjacent anionic phosphate oxygen, and thus assists Gua to be in a syn position. From the simulation, the average root-mean-square deviation of all the DNA heavy atom coordinates from the X-ray data is 0.99 Å. The bases are less deviated from the X-ray positions than the phosphates. The temperature factors from the simulation are consistent with those from the X-ray refinement, showing that the phosphates are more mobile than the bases.

Introduction

Since the first demonstration of the right to left handed transitions in DNA[1], significant developments in understanding of double-helical DNA conformations have been achieved[2-6]. The transitions strongly depend on counterions and solvents[7-9]. Unfortunately, present experimental techniques can hardly locate counterion binding sites in biomolecules except for a few cases which detected one or two counterions in especially well-ordered crystal systems[5,10]. On the other hand, a number of experimental studies on hydration structures have been reported. For small and well-packed crystal systems, most water molecules were found to be reasonably well ordered[11]. However, in most cases, only water molecules well bound in the first hydration shell have been located.

To understand the solvent and counterion effects in biomolecules, quite a few theoretical approaches have been studied[12-18]. But, microscopical understanding is not yet well understood. Computer simulation studies of hydration structures in macromolecular systems have shown serious multiminima problems, showing strong dependency of the results on the initial coordinates of water molecules. In particular, counterions in macromolecular systems can hardly move from one minimum to another one[15,19]. Since most counterions are missing in experimental data, it is very important to locate the most energetically favorable positions of ions before performing computer simulations. To scrutinize such possible positions, we utilize local energy analysis.

This work has been undertaken to help understand the stability of Z-DNA crystals in terms of the roles of water and counterions. Our study is based on a Z-DNA crystal of d(5'OH-5BrC-G-5BrC-G-5BrC-G-3'OH) grown in solutions containing sodium cacodylate and sodium chloride[6,20]. Figure 1 shows the structures of (a) the Z-DNA oligomer and (b) the crystal taken from the X-ray data. The crystal is a hexamer with 12 base pairs and 10 phosphates per asymmetric unit, exhibiting the symmetry of space group $P2_12_12_1$ with lattice constants of 17.9, 30.8 and 44.7 Å. The crystal structure is in hexagonal form where oligonucleotides are completely sheaded in water with counterions, without direct contacts between the oligomer nucleotides. The apparent contact between the hexamer nucleotides does not actually exist because the two parts of the molecule that appears to be in

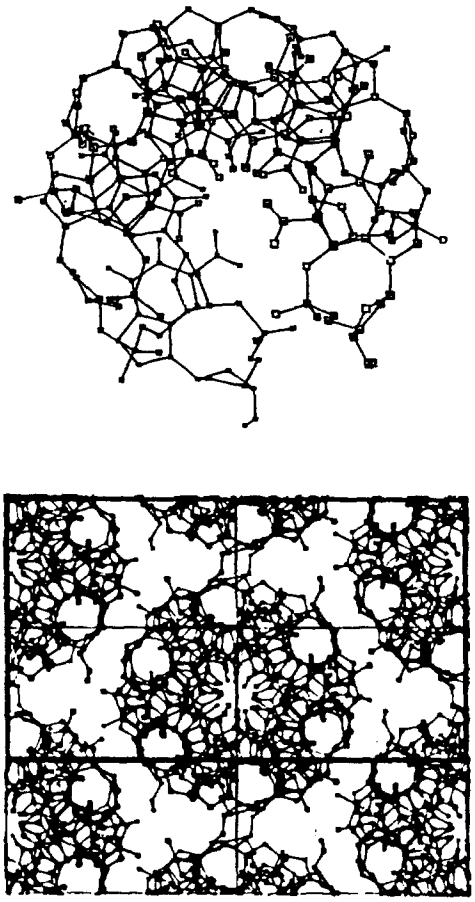

Figure 1. The crystal structure of d(BrC-G-5BrC-G-5BrC-G) in the x-y projection: (a) one oligomer and (b) one and a half unit cell of the crystal.

Table 1. Base Pairing of an Asymmetry Unit

5'O-C1--P2--G2--P3--C3--P4--G4--P5--C5--P6--G6--3'O
: : : : : :
: : : : : :
3'O-G12-P12-C11-P11-G10-P10-C9--P9--G8--P8--C7--5'O

contact are separated by as much as half the unit cell. Table 1 shows the hydrogen bonding base pairing of an asymmetry unit. To distinguish four asymmetry units in a unit cell, three non-primary asymmetry units will be denoted by adding suffixes of b, c and d to the primary asymmetry unit (*e.g.*, P8d with respect to P8). Suffix a denotes an asymmetry unit which is translated from the primary asymmetry unit by the unit cell length.

To have the charge balance in the crystal, there require 10 counterions per asymmetry unit. Since the X-ray data assumes two possible ion positions, eight counterions are still missing. Note that X-ray experiments have difficulties in distinguishing between water molecules and counterions. Therefore, we need to investigate the possible positions of sodium counterions by the local energy analysis. Then, using molecular dynamics (MD) simulations, we investigate more realistic positions of counterions and water molecules alongwith the DNA structures in the crystal. While most previous theoretical works have studied on oligomer for the simplicity, the present work investigates, in particular, the crystal symmetry effect, finding intermolecular bridging roles of counterions alongside water.

Method

Preliminary Search for Possible Positions of Counterions and Water Molecules. To locate the possible positions of counterions in the crystal, we calculated the interaction energies of one ion interacting with all Z-DNA atoms in the crystal at all points in a cubical grid with a square mesh of about 0.4 Å. The same method were used to locate well-bound water molecules. For water molecules, the oxygen atoms were located at each grid point, and then the energies were calculated at the most energetically favorable orientations. The energies lower than a certain threshold (*e.g.*, −5 kcal/mol for water) were stored for analyses. By comparing the energies at each grid point with those at the neighboring points, the local minima were located. Then, more refined local minima were searched for by comparing the interaction energies at more refined grids with a square mesh of 0.1 Å. If the neighboring local minima were within 2.0 Å, the higher energy minima were discarded.

The analytic interaction energy of the Z-DNA atoms with an ion or a water molecule was calculated with the following equation:

$$E=\sum_{ij}\{\epsilon_{ij}^{*}[(\sigma_{ij}^{*}/R_{ij})^{12}-2(\sigma_{ij}^{*}/R_{ij})^{6}]+Q_iQ_j/\varepsilon R_{ij}\}$$

where the energy is represented as the sum of pair potentials including the 6-12 potential terms and coulomb terms with distance-dependence dielectric constant ε. Most of van der Waals minimum radii (ϵ^{*}), well depths (σ^{*}), and charge (Q) were chosen from the values of Weiner *et al.*[21]. The van der Waals minimum radius and well-depth for Na^{+} used 1.6 Å and 0.05 kcal/mol, which were estimated from the *ab initio* potentials between Na^{+} and gramicidin[22] and between Na^{+} and phospholipid[23]. The coordinates of the heavy atoms in Z-DNA were fixed with the X-ray data. Only hydrophilic hydrogen atoms (*e.g.*, N-H and O-H) were attached with standard orientations. The dielectric constant ε was chosen with $1/\{1-(R_{ij}/R_c)^2\}^3$, where R_c is that cut-off length of 8 Å; thus, the potential function was smoothly cut-off[24].

Molecular Dynamics Simulation. The methodology of molecular dynamics used here is the same with that in our previous paper[24]. We used PMDAP (Postech Molecular Dynamics and Analysis Program) package which was written by one of the authors (K. S. Kim). For Z-DNA simulations, we used the periodic boundary condition for a unit cell which contained four Z-DNA asymmetric units, 40 counterions, and 308 water molecules. Intentionally without imposing the symmetry $P2_12_12_1$ in the unit cell, MD simulations were performed for 30 pico seconds (ps). The last 10 ps tajectory was analyzed for ensemble average.

Possibble Counterions

When DNA molecules are in solution, most counterions are solvated in water rather than bound to the DNA atoms except for high salt concentration. On the other hand, in crystal, a number of counterions cannot be well solvated by water because of small empty spaces arisen from packed DNA atoms; thus, only partially hydrated counterions tend to be bound to the most energetically favorable positions. The numbert of water molecules hydrating a counterion in biomolecules is limited to only few[14,15]. The interactions of ions with water molecules are much smaller than the interactions of ions with charged sites. Therefore, ions often have tendencies to move around near oppositely charged sites, and can often be bound directly to strongly nucleophilic sites. Such cases with binding sites have been shown in DNA crystals with high resolution X-ray experiments[6]. Although Gessner *et al.*[5] reported a case of indirect binding of Mg^{2+} *via* hydrated water to Z-DNA atoms, even this case shows that the hydrated ion is bound to nucleophilic sites.

However, due to disordering of counterions and water molecules, and also due to mobility of small counterions, most binding sites for alkali atoms can hardly be seen in DNA crystals, but will be near to nucleophilic sites. Serious static disordering is expected from the experiment of Drew *et al.*[25] which showed that the temperature factors of a B-DNA crystals at 16 K were enormously high. Such static disordering was also reported in the crystal of the left-handed hexamer which is being studied here[6]. If the interaction energy of an ion is very large at a certain position and if the ion is easily accessible to that position, the disordering of the ion may not be significant. However, when the two factors are somewhat incompatible, the ion can be statically disordered. Nevertheless, the stronger interaction at a certain position will help access of the ion to that position except for extreme occasions such as a totally internal pocket at rather low temperature.

In order to understand the role of counterions and water in Z-DNA crystals, we first investigate the X-ray structures. The distances between phosphate groups in Z-DNA crystals are much shorter than those in B-DNA and A-DNA crystals.

Table 2. Possible Positions of Na^+

	Local energy analysis			Molecular dynamics simulation		
	E(A)	Nearest Z-DNA atoms		E(A,W,I)	[occur] Nearest Z-DNA atoms	#Wat
1	−142	P8d-OB(2.3)	P3-OB(2.3)	−48, −79, 27	[3]P8d-OB(2.3) [2]P3-OB(2.3)	4.0
2	−142	P10-OB(2.2)	P5d-OA(2.3)	−69, −63, 28	[4]P10-OB(2.4) [4]P5d-OA(2.4)	4.0
3	−139	P9-OB(2.4)	G6d-O5'(2.6)	−53, −96, 43	[2]P9-OA(2.4) [2]P8-OA(2.3)	5.2
		P9-OA(2.9)	(P6d-OA(3.3))		[1]P6d-OA(2.6)	
4	−126	P3-OA(2.3)	P4-OA(2.3)	−19, −118, 28	[0]	6.7
4'	−111	P4-OA(2.3)	(P4-OB(3.1))			
4"	−110	P2c-OB(2.4)	(P3c-OA(2.7))			
5	−121	P12-OB(2.2)	P11-OA(2.3)	−48, −65, 12	[4]P12-OB(2.4) [1]P11-OA(2.3)	4.3
5'	−117	P11-OA(2.5)	P10-OA(2.5)			
6	−116	P2c-OB(2.3)	P8d-OA(3.2)	−25, −130, 48	[0]	6.2
6'	−108	P2c-OB(2.4)	P2c-OA(2.6)			
7	−111	P5-OB(2.5)	P4-OB(2.9)	−35, −113, 42	[2]P5-OB(2.3)	6.0
8	−108	P6-OA(2.3)	P6-OB(2.4)	−16, −104, 12	[1]P6-OB(2.4)	5.0
9	−95	P11-OB(2.2)	G2a-N7(2.4)	−33, −84, 18	[3]P11-OB(2.3) [1]G2-N7(2.4)	5.2
10	−73	G10-N7(2.5)	G12c-N7(2.5)	−17, −104, 16	[0]	5.8
		(G10-O6(3.4)	G12c-O6(3.5))			
11	−94	P12c-OA(2.3)				

E(A), E(W) and E(I) denote interaction energies of an ion (Na^+) with DNA atoms, water, and ions, respectively. The average value of the sum of E(Å), E(W) and E(I) is −104 kcal/mol. [occur] denotes the number of occurrences appeared among four asymmetry units in a unit cell. #Wat denotes the number of water molecules solvating an ion in the first solvation shell. Distances are in Å and energies are in kcal/mol. Distances from ions to the nearest atoms are in parentheses.

For the Z-DNA crystal investigated here, the distances between neighboring phosphates in the same strand are only ~6 Å and those between neighboring phosphates in different asymmetry units are ~7 Å except for the beginning and ending phosphates. The distances of double-bonded anionic oxygens of the same phosphates are 2.4-2.7 Å; thus, the distances between two oxygens in different phosphate groups are only 4.0-5.3 Å for both the adjacent lower and higher sequence bases, and 4.5-5.5 Å for non-adjacent sequence bases. Further, these anionic oxygens are often neighbored by anionic atoms, O1', O3', O5' or N7. Once counterions can stabilize these anionic oxygens in two different asymmetry units, two oligomers can be bound to form the crystal. To understand such roles of counterions in the crystal, local energy analysis was performed. By scanning the whole space of the crystal (actually one quarter of the unit cell due to the crystal symmetry), the most probable counterion positions were located. Table 2 lists the possible binding positions of counterions, the energies of which are lower than −70 kcal/mol. These minima are grouped into 11 regions. It is because a few positions (such as 4, 4', 4") are somewhat near to each other or have common nucleophilic binding sites. The local minima are marked in Figures 2 and 3. Figure 2 shows the *iso*-energy maps (x-y projection) of Na^+ interacting with the whole Z-DNA molecules in the crystal. Figure 3 is the cylindrical *iso*-energy maps for Na^+. As shown in Table 2 and Figures 2 and 3, most counterions bind phosphate anionic oxygens in the same strand or two different asymmetry units.

To obtain a more realistic conclusion, MD simulations were performed. For the initial counterion positions, we used Na^+ positions, 1 to 10 which are located by the local energy analysis. Position 11 was discarded because position 10 was conjectured as NH_4^+ from the X-ray data. From 30 ps MD simulations, the last 10 ps trajectory was analyzed. As shown in Table 2, 18 out of 40 counterions in one unit cell moved away from the Z-DNA nucleophilic sites so as to be fully hydrated, though the ions were still near those sites. The total number of binding sites that counterions are directly bound to are 30; thus, the average number of direct binding sites per counterion is 0.75. The average number of water molecules directly bound to a counterion is 5.25. The average coordination number for a counterion is 6.0. When Na^+ is solvated in bulk water, the pair distribution function of the distance between Na^+ and water oxygen has the maximum occurrences at 2.2 Å. But, in Z-DNA crystal, the maximum peak appears at 2.45 Å. This is expected because solvation of Na^+ is somewhat hindered in presence of phosphate anionic oxygens in the crystal.

Hydration Structures

Simulation studies of biomolecules have shown that water structures are film-like, bound to strongly hydrophilic sites[14,15]. The possible water molecules predicted by the local energy analysis are strongly bound to binding sites without perturbing much by neighboring water molecules. Indeed, these computed water positions are in good agreement with X-ray data. The binding sites which water molecules are bound to with strong interaction energies are Gua-N2, Cyt-N4, Gua-N7, Gua-O6, and P-OA/OB. As shown in Table 3, water molecules are well bound to Gua-N2. These water positions predicted by the local energy analysis are in excellent agreement with the X-ray positions. These water molecules help

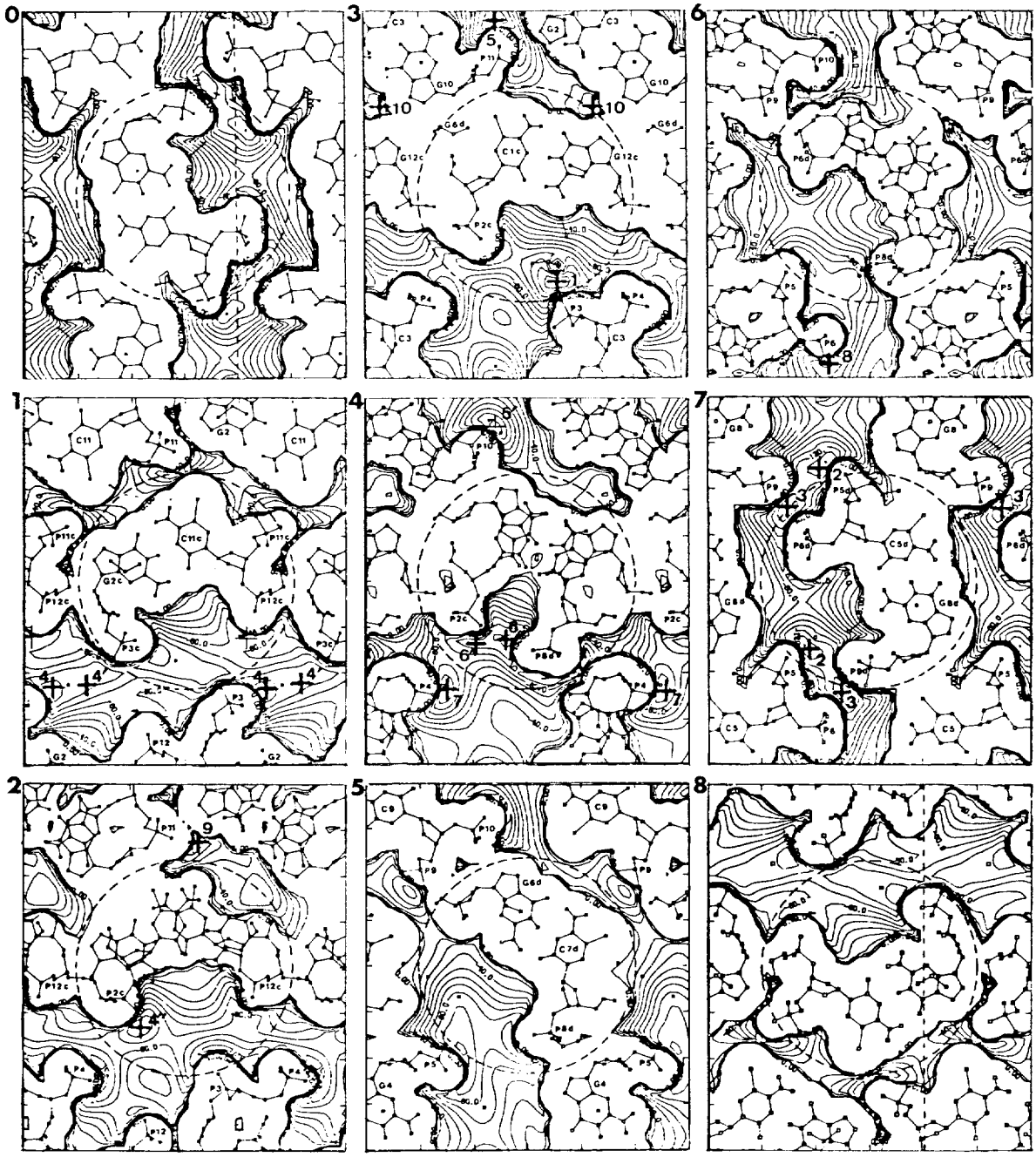

Figure 2. *Iso*-energy maps for the interaction energy of Na^+ in the crystal on the planes of (0) $z=-11.18$, (1) $z=0.00$, (2) $z=1.86$, (3) $z=3.73$, (4) $z=5.59$, (5) $z=7.45$, (6) $z=9.32$, (7) $z=11.18$, (8) $z=22.37$ or (-22.37) Å. Each inset covers the area of 14.5×30.8 Å^2 (i.e., $1.5a\times1.0b$ in lattice constants, or 1.5 unit cell cross section). The area of one unit cell in the x-y projection is on the left side of the dotted lines in inset (0) or (8). One examplary cylindrical volume is in the center of the circles drawn in each inset, the size of which is almost comparable to the size of one hexagonal primitive cell. The radius of the circle is 9 Å. Contour line intervals are 10 kcal/mol. The molecular structure in each inset includes the portion of Z-DNA molecules between $z-1.5$ Å and $y+1.5$ Å for the given plane. Positions of Na^+ in Table 2 are marked by crosses.

bridge Gua-N2H and phosphate anionic oxygens OA/OB, while counterions tend to be either partially or fully solvated near OA/OB and screen charges so as to put other anionic phosphates nearby. Our MD simulation shows that one half of these water molecules are well bound to Gua-N2, and a few of them are partially bound to phosphate anionic oxygens. Although the bridging role is not perfectly consistent in all regions near six Gua-N2 atoms, this bridging role is important in that water stabilizes the internal structure of an oligomer alongwith counterions. Indebted from the effect of counterions attracting the anionic phosphates, the intra-structure stability due to water helps Gua be in a syn position for Z-DNA in contrast to the case that Gua is in an anti position for B-DNA.

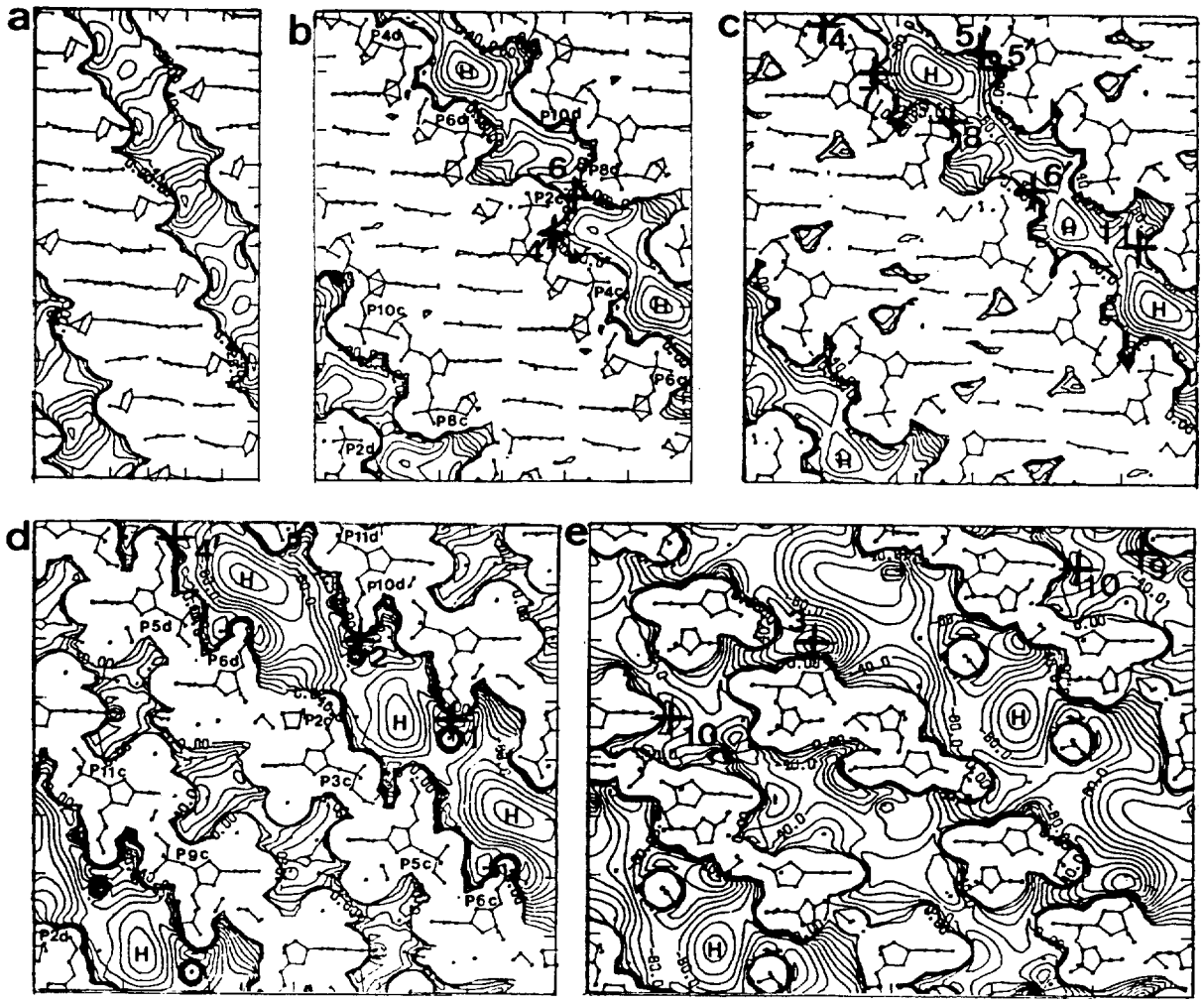

Figure 3. Cylindrical *iso*-energy maps for the interaction energy of Na^+ in the crystal with radii of (a) $R=3.38$, (b) $R=5.63$, (c) $R=6.75$, (d) $R=7.88$ and (e) $R=9.00$ Å . Each inset covers the area of $2\pi R\times 30.8$ Å^2. Contour line intervals are 10 kcal/mol. The molecular structure in the insets includes only a portion of Z-DNA molecules between $R-2.5$ Å and $R+2.5$ Å for the given cylindrical plane. Na^+ positions in Table 2 are marked by crosses. Notation H in the figure denotes a local maximum, though the potential is attractive. The helical structures of DNA strands and major/minor grooves can be easily noticed in these maps.

Table 3. Distances from Water Oxygens Bound to Gua-N2H to Atoms −N2 and P-OA/OB (in Å)

	X-ray	local	MD	[occur]		X-ray	local	MD	[occur]
2-N2	3.1	3.3	3.2[2]		3-OA	3.0	3.0	3.0[1]	
4-N2	3.1	3.3	3.0[2]		5-OB	3.0	3.0	[0]	
6-N2	3.2	3.2	3.2[1]		6-OB	3.1	3.0	2.9[1]	
8-N2	2.9	3.4	3.4[2]		9-OA	3.3	3.0	[0]	
10-N2	3.4	3.8	3.0[4]		11-OA	3.4	3.1	3.8[2]	
12-N2	3.1	3.4	3.0[2]		12-OA	3.8	3.1	[0]	

"local" denotes the local energy analysis. Refer to the footnote in Table 2 for [occur].

The local interaction energies of water molecules bridging Cyt-N4 and Gua-N7 are slightly smaller than water molecules adjacent to Gua-N2, and the agreement with the X-ray data is less conspicuous than water molecules adjacent to Gua-N2. Nevertheless, those water molecules contribute to inter-molecular stability, The interaction energies of water molecules near OA/OB are similar to those in Gua-N2, but the potential well near that site is so wide that the localization is not easy. Thus, five to seven water molecules hydrate the phophate oxygens in the simulation.

Protein Structures

From the MD simulation, we have obtained the average root mean square deviations (rms) of atomic coordinates from the X-ray data, and the average rms fluctuations of atomic coordinates with respect to the average positions. The average rms deviation of all the DNA heavy atom coordinates is 0.99 Å. The values of the bases, sugars and phosphates are 0.86, 1.02, and 1.23 Å, respectively. Consequently, the bases are less deviated from the X-ray positions than the phosphates. The average dynamic rms flucatuations of all DNA heavy atoms, counterions, and water molecules are 0.68, 0.98 and 1.00 Å, respectively. Including the static disordering, the average rms fluctuation of all DNA heavy atoms is 0.83

Å; thus, the temperature factor is 18 Å^2. These values can be compared with 0.59 Å and 9.2 Å^2 of the X-ray refinement[6]. The average rms fluctuations of the bases, riboses, and phosphates are 0.78, 0.83, and 0.97 Å, respectively; thus, their temperature factors are 16, 18, 25 Å , respectively. This shows that the phosphates are more mobile than the bases. The corresponding values from the x-ray refinement are 0.53, 0.58 and 0.76 Å, respectively, and thus, 7.3, 8.9, 15.2 Å^2, respectively. For the rms fluctuations or temperature facors, our simulation results are somewhat larger than those from the X-ray refinement. But, there exists a good consistency between the simulation and the X-ray.

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Template Synthesis and Characterization of Binuclear Nickel(II) and Copper(II) Complexes of Double-ring Macrocyclic Ligands

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New binuclear Ni(II) and Cu(II) complexes with various alkyl derivatives of 1,2-bis(1,3,6,8,10,13-hexaaza-1-cyclotetradecyl) ethane, in which two fully saturated 14-membered hexaaza macrocyclic subunits are linked together by an ethylene chain, have been synthesized by the one step template condensations of formaldehyde with ethylenediamine and appropriate primary alkyl amines in the presence of the metal ions. Each macrocyclic subunit of the double-ring macrocyclic complexes contains one alkyl pendant arm and has a square planar geometry with a 5-6-5-6 chelate ring sequence. The visible spectra and oxidation properties indicate that the metal-metal interactions of the binuclear complexes are not significant. Synthesis, characterization, and the properties of the complexes are presented.

Introduction

There has been considerable interest in the synthesis of binuclear macrocyclic complexes, since the complexes often represent a helpful tool in the study of metal metal interactions and multi-metal centered catalysts.[1-17] In order to obtain