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Comparison of a Left-Handed Z-DNA Molecular Structure Determined by X-Rays with That Simulated by a Molecular Dynamics

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The 1.0 Å resolution X-ray crystal structures of the left-handed Z-DNA(Z-I and Z-II conformations) were compared with that of the simulated molecular dynamics(MD) structure both in vacuo and in solution. Whilst the X-ray structure showed a tendency for the $d(CG)_3$ molecule to take on a Z-II conformation in high salt solution or in strongly ionized conditions, the MD simulation with Na^+ ion for 30 ps revealed that the left-handed $d(CG)_3$ structure with the Z-I conformation was transformed into the Z-II conformation in the torsion angles of the C3, G4 and C5 phosphate groups, and furthermore, when K^+ ion was used as the counterion instead of Na^+ ion, the torsion angles in almost the entire $d(CG)_3$ molecule were preserved. On the other hand, the MD calculation resulted in some very important changes on the sugar puckerings; the simulation with Na^+ ion indicated that all the sugar puckerings of cytosine residues were changed from C2′-endo to C3′-endo, while those for guanosine residues tended to keep unchanged (C3′-endo) except for a terminal residue (G6). © 1997 Academic Press

Since the publication of the Watson and Crick double helical DNA structure[1], it has always been the belief that DNA existed in a right-handed conformation. This was however shown not to be true with the publication of the structure of the left-handed DNA oligomer $d(CG)_3$ by Rich et al.[2] and Dickerson et al.[3]. In our reports on the crystal structures of complexes of the left-handed oligomer $d(CG)_3$ and Polyamines[4-7], we described that the Z-DNA existed in two conformations, i.e., the Z-I and Z-II conformations(Table 1) according to the differences in the backbone torsion angles(Fig.1). Similar conformations have also been reported by Gessner et al.[8]. These studies indicated that in solution, the $d(CG)_3$ conformation converted from the right-handed B-form to the left-handed Z-form adopting the C2′-endo and C3′-endo sugar puckerings in cytosine and guanosine residues, respectively, in increasing concentrations of salt or alkali metal ions [9-10]. Theoretical simulations of the conformation of the DNA using semiempirical energy calculations and molecular dynamics(MD) have shown that the spermine bound to the major groove of the B-DNA for stabilizing the conformation by maximizing the interactions between the proton acceptors on the DNA oligomer and the proton donors on the spermine molecule[10]. Furthermore, there was a tendency to rotate the torsion angles around most of the $d(CG)_6$ phosphates in aqueous solution with K^+ ion in accordance with a Z-I → Z-II transition[11]. The ionic strengths of the added ions may affect the Z-I → Z-II transition. We have since been interested to know whether the left-handed DNA may exist in nature in reality, and under what conditions the Z-I → Z-II transition or the left-handed Z-DNA → the right-handed B-DNA transition may occur. This communication deals with the comparison of the MD simulated structure of Z-DNA with those determined by X-rays.

MATERIALS AND METHODS

Crystallization and X-ray structure analysis. The DNA hexamer was co-crystallized with the various polyamines and the crystal structures were determined as previously reported[4-7]. Briefly, the crystallization condition was; 2 mM ammonium salt of $d(CG)_3$ in 30 mM sodium cacodylate buffer (pH 7.0) containing 15 mM $MgCl_2$ and 10 mM polyamine trichloride or tetrachloride was crystallized by the vapor diffusion method. All the refined cell constants of the complexes were isomorphous to that of the first-determined Z-DNA, $d(CG)_3$ crystal, with the space group $P2_12_12_1$[2]. The integrated intensities were measured by the continuous ω-scan method with a monochromatic Cu-Kα radiation using the Nicolet P3 automated four-

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TABLE 1

Crystal Structure Values of the Back-bone Torsion Angles (The Values Are Given in Degrees)

	Z-I-DNA		Z-II-DNA		
	pC	pG	pC	pG	B-DNA
α	223	47	146	92	314
β	221	179	164	193	214
γ	56	191	66	157	36
δ	138	99	147	94	156
ϵ	266	256	260	181	155
ζ	80	291	74	55	264
χ	201	68	212	62	260

Data for B-DNA from fiber diffraction. The underlined torsion angles are used to distinguish between Z-I and Z-II conformations.

circle diffractometer at l0°C. The intensity data was collected up to the l.O Å resolution with a scan speed of 4°/min and applied the semiempirical absorption correction[12]. The phases of each complex were solved by the molecular replacement method with atomic coordinates of d(CG)$_3$[2] and the structure was refined by least squares method [13] and the molecular dynamics (program:X-PLOR[14]). The d(CG)$_3$ molecules were traced on a 2[Fo]-[Fc] map calculated with the CCP4 program[15]. The model building was performed with the program O[16] on an IRIS Indy Workstation and the structures were refined to a residual R-values from 0.161 to 0.191 with an averaged r.m.s. deviation from 0.16 to O.30 for the atomic coordinates. The detailed procedures for structure determination were shown in the references[4-7]. The numbering of the residue in d(CG)$_3$ is shown in the Scheme 1.

Molecular dynamics(MD) calculation. The MD study was performed both in vacuo and in solution by using the average bond lengths and torsion angles calculated with the atomic coordinates of the left-handed Z-form d(CG)$_3$ duplexes found in the crystal structures of three d(CG)$_3$:polyamine complexes[5-7] as the starting model.(Fig.2). In the first step (in vacuo), the d(CG)$_3$ molecule was optimized with 150 iterations using the steepest descents and conjugate gradients methods with a force constants of Amber Force Field [17]. The energy is constrained in 100.0 Kcal/Å^2 until the maximum deviation is less than 1 Kcal/Å^2. Initialized dynamics was carried out with 2000 iterations for 2 ps with the following parameters:overlap 0.017 Å, cutoff 13.0 Å, cutdis 12.0 Å, swtd 1.5 Å and additional automatic parameters. The d(C-G) base-pairs were constrained by hydrogen bonding with 20-30 Kcal/mol. The simulation was continued for a further 30000 iterations for 30 ps in vacuo without counterions. As the second step (in vacuo), neutralization of the ten negative charges of the d(CG)$_3$ phosphate groups was accomplished by the addition of ten Na$^+$ or K$^+$ ions which were chosen because of its comparatively weak ions. In order to overcome the difficulty concerning the description of the counterion atomsphere, the effective charge screening of the phosphate groups due to the counterions was incorporated and these ions were located at 5 Å from the phosphorous atom and bisected the angle Ol-P-O2 as shown in the Scheme 2. As the third step, the MD simulations were performed in aqueous solution without the constrain on the base pairs. The dielectric constant in the close vicinity of the d(CG)$_3$ helix is rather different from that of bulk water and we introduced distance-dependent dielectric constants. 776 water molecules were randomly generated. The positions of Na$^+$ or K$^+$ cations, the parameters and the iterations of the MD simulation were the same as these for the simulation in vacuo(Table 2). The r.m.s. deviations of the atomic coordinates for the d(CG)$_3$ dulex and Na$^+$ ions after 30 ps simulation were 2.424 Å and 5.074 Å, respectively, and that for the d(CG)$_3$ and K$^+$ ions were 1.882 Å and 3.941 Å, respectively. These calculations were carried out with the program DISCOVER[19] and performed on an IRIS Indy workstation at Osaka University of Pharmaceutical Sciences, on an ACOS-3700 System at Research Center for Protein Engineering, Institute of Protein Research, Osaka University, and

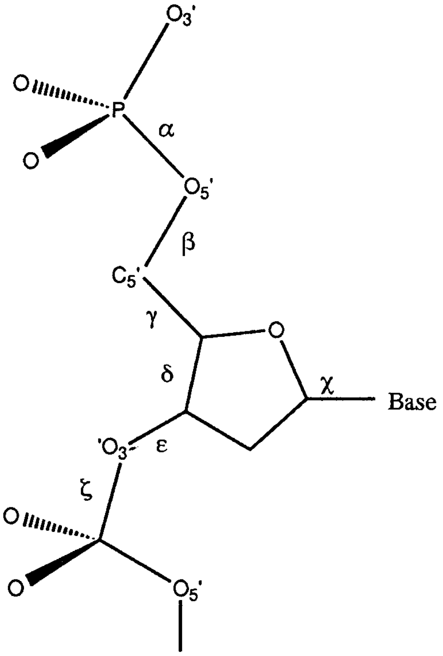
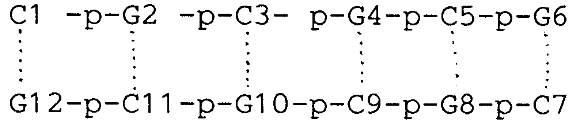


FIG. 1. Definition of the backbone torsion angles.



SCHEME 1

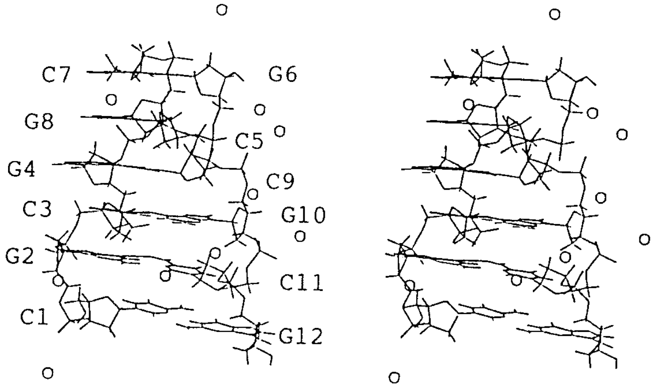
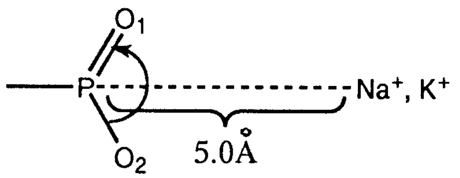


FIG. 2. Starting model for the left-handed d(CG)$_3$ duplex using the average torsion angles and bond lengths found in the crystal structures of three d(CG)$_3$:polyamine complexes[5–7].



SCHEME 2

then on an IRIS Indigo-2 workstation at Fujisawa Pharmaceutical Co. Ltd., Osaka.

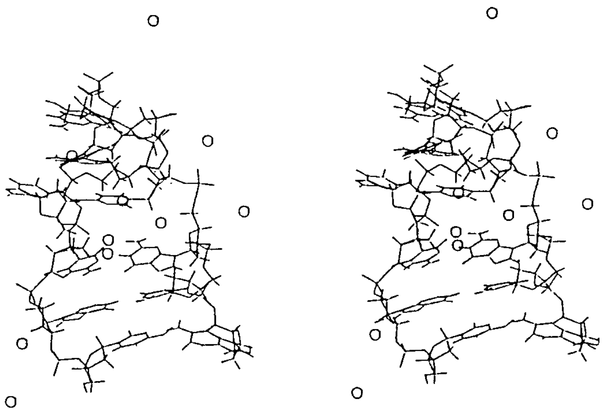


FIG. 3. Molecular conformation of left-handed $d(CG)_3$ duplex the sodium counterions after 30 ps molecular dynamics calculation

RESULTS AND DISCUSSION

The differences between the Z-I and Z-II left-handed DNA appear in the particular backbone torsion angles, α and β for cytosine nucleotide residues, and ϵ and ζ for guanine nucleotide residues, as shown in Table 1. According to our results on the crystal structures of $d(CG)_3$ + spermine(SP, form) [5], $d(CG)_3$+N-(2-amino)-2,4-diaminobutane (PA(24) form)[6], and $d(CG)_3$+ spermidine (SPM form)[7], the torsion angles of G4 residue belonged to Z-II type in all the structures, and those of C8 residue in SPM form were exactly Z-II type, but those in PA(24) and SP forms were not completely Z-II type, but rather intermediate. These results suggested that the Z-I → Z-II transition might be dependent on the ionic strength around the $d(CG)_3$ duplex, because the ionic strength of polyamine increases in order of spermidine, N-(2-amino)-2,4-diaminobutane and spermine[20]. At the biginning of the MD simulation, the starting model(Fig.2) was optimized to form an idealized Z-I type $d(CG)_3$ duplex. As the first step, the MD simulation in vacuo was performed with hydrogen bond constrain of about 20-30 Kcal/mol for the base-pairs, but it failed and the molecular conformation of $d(CG)_3$ molecule was stabilized as a ladder-like structure but not helix. As the second step, the MD calculation was carried out in vacuo with counterions, but this calculation failed too, and helix was broken. As the third step, the MD simulation in aqueous solution without hydrogen bond constrains for the base-pairs was calculated, and in this case, the left-handed helical conformation of $d(CG)_3$ duplex was preserved without disruption of the base-pairs. As the results, the initial energy of MD with Na^+ counterions was −12940 Kcal/mol and the final energy was −14030 Kcal/mol which means the conformational stabilization, while the initial and final

TABLE 2

Data of the MD Simulation Z-DNA + Sodium Cation

No. of water molecules	776
No. of sodium counterions	10
Atomic charges	Full
Cutt off (Å)	13.0
Box dimension (Å^3)	30.0 × 28.0 × 35.0
Periodic boundary conditions	In all three directions
Algorithm for solving the equations of motion	Verlet-method [19]
Z-DNA + Potassium cation	
No. of water molecules	776
No. of sodium counterions	10
Atomic charges	Full
Cutt off (Å)	13.0
Box dimension (Å^3)	30.0 × 28.0 × 35.0
Periodic boundary conditions	In all three directions
Algorithm for solving the equations of motion	Verlet-method [19]

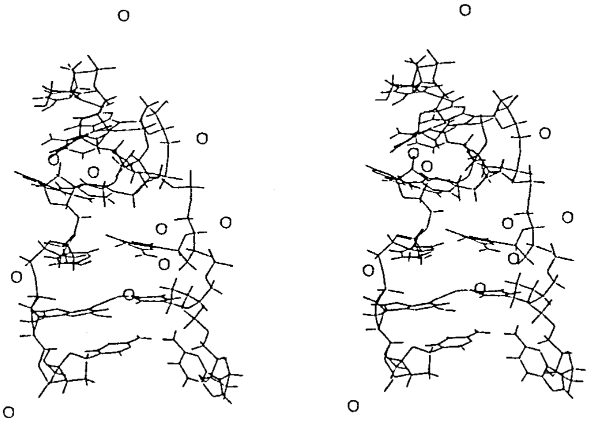


FIG. 4. Same as Fig.3 except with potassium counterions.

TABLE 3

Backbone Torsion Angles for Cytosine Nucleotide and Guanine Nucleotide Residues of Left-handed d(CG) 3 Duplex

	C1	C3	C5	C7	C9	C11	G2	G4	G6	G8	G10	G12
α												
X-RAYS		215	170		205	209	66	68	72	62	62	82
NDY		159	69		272	239	67	225	319	68	47	98
KDY		220	273		57	205	64	317	36	83	50	53
β												
X-RAYS		243	164		219	240	185	188	177	190	182	184
NDY		144	169		271	234	179	176	286	190	142	161
KDY		257	156		174	188	191	264	202	184	159	162
γ												
X-RAYS	48	49	40	47	56	53	176	181	182	176	180	184
NDY	198	148	153	57	291	57	184	67	216	154	190	172
KDY	194	49	36	195	269	91	173	206	155	168	189	224
δ												
X-RAYS	144	152	147	138	144	144	97	97	149	102	101	151
NDY	166	150	159	103	159	145	91	154	83	88	85	146
KDY	155	140	95	139	160	158	93	96	80	76	106	75
ϵ												
X-RAYS	268	259	266	263	266	259	241	185		242	242	
NDY	301	221	247	281	315	279	245	185		203	220	
KDY	288	271	308	280	296	287	210	272		244	234	
ζ												
X-RAYS	76	75	76	79	75	74	284	63		305	291	
NDY	85	91	164	84	87	86	309	13		357	289	
KDY	79	186	77	66	108	74	284	312		275	292	
χ												
X-RAYS	210	206	212	212	202	202	63	56	77	64	64	80
NDY	179	191	210	226	185	215	61	93	12	63	55	49
KDY	205	196	209	227	197	199	59	38	73	52	89	57

energies with K^+ ions were -12828 Kcal/mol and -13835 Kcal/mol, respectively. In this case, the cations bound to the terminal nucleotide residues of the $d(CG)_3$ duplex come apart from the surface of the helix but those bound to the central residues of the $d(CG)_3$ duplex come closer to the surface of the duplex, and the straight helical axis found in the crystal structures was kinked after 30 ps MD calculation as shown in Figs.3 and 4. The backbone torsion angles for each nucleotide residues are shown in Table 3, in which X-rays is the values used for the starting model (Fig.2), and NDY and KDY are the values after 30 ps MD calculation with Na^+ and K^+ counterions, respectively. As indicated in Table 3, the torsion angles of C3, G4 and C5 phosphate groups of the $d(CG)_3$ duplex were transferred from Z-I to Z-II, but that of G8 phosphate group was neither Z-I nor Z-II, but just an in-between, when the counterion was Na^+ cation. When the counterion was changed to K^+ cation, the ideal Z-I conformation of the $d(CG)_3$ duplex was roughly preserved, and this result was quite different from the data by Eriksson and Laaksonen[11] indicating the Z-I → Z-II transition in most of the nucleotide residues of the left-handed $d(CG)_6$ duplex. One of the most remarkable feature on the molecular conformation of the left-handed Z-DNA duplex is the sugar puckering, that is, C3′-endo puckering in purine nucleotide residue and C2′-endo puckering in pyrimidine nucleotide residue, while all the sugar puckerings in the right-handed B-DNA duplex are C2′-endo conformations. As shown in Table 4, when Na^+ ion was used as the counterion, the MD calculation for 30 ps revealed that all the sugar puckerings of the $d(CG)_3$ duplex were C3′-endo conformations irrespective of the base varieties except for a terminal G12 deoxyribose ring. On the other hand, after 30 ps MD calculation with K^+ cation, the sugar puckerings of the $d(CG)_3$ duplex were mainly C3′-endo conformations except for C5, C7, and G10 nucleotide residues with C2′-endo puckerings. The results mentioned above is too far to draw the satisfactory conclusion about the effect of the counterions upon the Z-I → Z-II transition of the left-handed $d(CG)_3$ duplex. Of many important factors controlling the Z-I → Z-II transition, the ionic radius and electronegativity as well as the ionic strength and dielectric constant should be considered, and then the MD simulation time, 30 ps, may still not be enough to get a stable conformation of the left-handed d$(CG)_3$ duplex, because 70 ps MD simulation was performed for the Z-I → Z-II transition of the d$(CG)_6$ duplex[11].

TABLE 4

The Sugar Puckring and P-Value

	C1	C3	C5	C7	C9	C11	G2	G4	G6	G8	G10	G12
X-RAYS	159 C2′-endo	149 C2′-endo	150 C2′-endo	148 C2′-endo	148 C2′-endo	150 C2′-endo	32 C3′-endo	23 C3′-endo	169 C2′-endo	34 C3′-endo	13 C3′-endo	166 C2′-endo
NDY	11 C3′-endo	4 C3′-endo	3 C3′-endo	0 C3′-endo	39 C3′-endo	4 C3′-endo	35 C3′-endo	14 C3′-endo	13 C3′-endo	68 C4′-exo (C3′-endo)	41 C4′-exo (C3′-endo)	179 C2′-endo
KDY	5 C3′-endo	1 C3′-endo	112 C2′-endo	141 C2′-endo	35 C3′-endo	4 C3′-endo	34 C3′-endo	9 C3′-endo	37 C3′-endo	42 C4′-exo (C3′-endo)	141 C2′-endo	14 C3′-endo

P-values: pseudorotation phase angles (degree) [21].

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