Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

X-ray crystallographic study of the possible binding sites of the monovalent cations in the Z-DNA structure

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Received 30 January 2003
Accepted 25 April 2003

Determination of the binding positions of light metal ions in the structure of oligonucleotides remains a challenge owing to their irregular coordination geometries, low electron densities and mobility in the crystals. To study the interactions between monovalent cations and DNA, the heavier alkali metal ion Rb^+ was used to replace Na^+ and K^+ in the Z-DNA crystal and the structure was analyzed using X-ray cryocrystallography. The resolution of the Rb-d$(CGCGCG)_2$ crystal data set is 1.76 Å, with an R_{merge} of 0.061. The final residual factor (R factor) for the crystal structure is 0.175. The positions of 16 Rb^+ ions were tentatively assigned, with most of them having partial occupancies. Three rubidium ions with full occupancies coordinated to two DNA double strands were located in the Z-DNA crystal. The results of this work suggest that there are numerous relatively low-energy sites for binding of monovalent cations to Z-DNA and that these sites are cation–water hybrid positions.

1. Introduction

To reveal the function of metal ions in the biological activity and structural stabilization of DNA, studies of the interactions between metal ions and DNA (or RNA) using X-ray crystallography have been carried out by many research groups. The binding positions of the divalent cations, including Mg^{2+}, Ba^{2+}, Co^{2+} and Cu^{2+}, have been determined with the help of their regular coordination geometries (Gessner *et al.*, 1985; Gao *et al.*, 1993). On the other hand, it is difficult to identify the binding positions of monovalent cations (Na^+ and K^+) in nucleic acid crystals owing to their irregular coordination geometries and lower electron densities. Shui and coworkers proposed a hybrid solvent model for the solvent structure around DNA, in which solvent sites are occupied by water–cation hybrids (Shui *et al.*, 1998). Anomalous diffraction from K^+, Rb^+, Cs^+ (Tereshko *et al.*, 2001) and isomorphous difference Fouriers of Tl^+ (Howerton *et al.*, 2001) were used to locate the binding sites of these cations in A-DNA and the B-DNA crystals. The results showed that most observed monovalent cation sites in the DNA crystals are partially occupied positions. In this study, the rubidium ion, a heavier alkali metal ion with an ionic radius of ~1.5 Å and a typical $M^+\cdots O$ distance of 2.8 Å, which are similar to those of K^+ (ionic radius of 1.3 Å and $M^+\cdots O$ distance of 2.7 Å) was used to replace Na^+ and K^+ in the Z-DNA crystal in order to study the interactions between monovalent cations and Z-DNA. The crystals were grown in a solution containing the oligonucleotide d$(CGCGCG)_2$ and a high concentration of rubidium chloride. Data to 1.76 Å resolution were collected at cryogenic temperature. The structure was analyzed to detect the binding sites of Rb^+ ions in the Z-DNA crystal. The results of the tentative assignment of the binding positions of Rb^+ ions in the Z-DNA structure are presented in this paper.

2. Experimental

A DNA hexamer with sequence CGCGCG was synthesized using the phosphotriester method and purified by reversed-phase HPLC. The crystals were grown in Linbro plates (Hampton Research) at ambient temperature by the sitting-drop vapour-diffusion method. The crystallization drops contained 4.2 mM oligonucleotide, 40 mM rubidium cacodylate pH 7.0, 200 mM rubidium chloride and 5% 2-methyl-2,4-pentanediol (MPD). The equilibrating solution contained 300 mM rubidium chloride and 60% MPD. Crystals with the shape of pseudo-hexagonal blocks (Fig. 1) grew in 3–6 d. The dimensions of good crystals were approximately 0.5 × 0.4 × 0.2 mm.

For data collection, a crystal was picked up from the crystallization drop with a rayon loop (Hampton Research) and flash-cooled with liquid propane. The crystal was maintained at 83 K during the data collection with an Enraf–Nonius 558S cryostat. The data were collected with a MAR Research image plate using Cu $K\alpha$ radiation (λ = 1.5418 Å) generated from an Enraf–Nonius diffractometer. The distance between the crystal and image plate was 70 mm. A data set with a total rotation of 90° in φ with increments of 1.5° in each scan

was collected. The data set has a total of 2556 unique reflections, with 2474 reflections having an intensity larger than 2σ.

The image files were processed with the *DENZO* program (Gewirth *et al.*, 1995) and reduced with *SCALEPACK* (Gewirth & Otwinowski, 1995). The resolution of the data set is 1.76 Å, with an $R_{\rm merge}$ of 0.061. Both $R_{\rm linear}$ and $R_{\rm square}$ are less than 0.11 for the overall data set. Over 90% of the reflections were obtained to 1.76 Å resolution. The data were reliably measured, with two thirds of the reflections in the high-resolution shell (1.83–1.76 Å) having an $I/\sigma(I)$ of 10 or greater.

Since the unit cell of Rb-Z-DNA is similar to that of the Mg-Z-DNA structure (Gessner *et al.*, 1989), the molecular-replacement method was employed for structure determination using the Mg-Z-DNA structure (without Mg^{2+} ions) as the initial trial model. The starting structure (including all solvent H_2O molecules) was refined with the *X-PLOR* program (Brünger, 1992) against Rb-Z-DNA data. Positional refinement, individual *B*-factor refinement and simulated annealing were carried out iteratively for the structure refinement. Electron-density maps ($2F_o - F_c$ and difference $F_o - F_c$) were examined with the *CHAIN* program (Sack, 1988). Solvent molecules were deleted if they had very high *B* values and there was negligible $2F_o - F_c$ density at their positions. New water molecules were added to the peaks of the corresponding sum and difference density. The positions of Rb^+ ions were assigned after careful examination of the electron densities, *B* factors and coordinations of the sites. The molecular structure of Z-DNA and coordination of Rb^+ ions were also inspected with the *CHAIN* program. For disordered structures, two partial occupied sites for each atom were assigned to alternate structures. The final residual factor (*R* factor) for the structure is 0.175. The crystal data and refinement parameters are listed in Table 1.

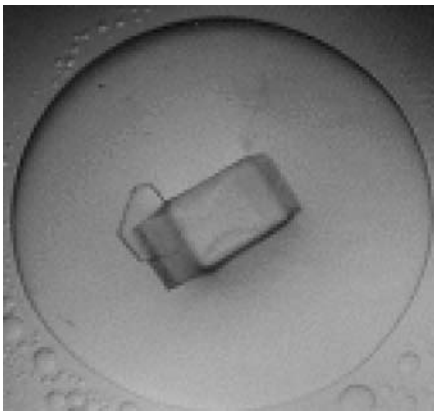

Figure 1
Rb-Z-DNA crystals.

3. Results and discussion

The DNA part of the Rb-Z-DNA structure is similar to the Mg-Z-DNA structure except that the phosphate group of cytidine 5 was found to be disordered with a partial Z_I/Z_{II} conformation. The disordered structure is revealed by very close electron-density peaks (with distance of 1.36 Å) near the position of the phosphorus site. Alternate conformations with half occupancy were assigned for the atoms in this phosphate group. After refinement, the *B* factors for the atoms in both groups are about 6.6 $Å^2$, indicating that the assignment of half occupancy for each is appropriate.

As with most alkali-metal atoms, rubidium is soft and does not have a regular hydration coordination geometry. The Rb—O distance is close to the H_2O hydrogen-bonding distance (about 2.8 Å). The Rb^+ ion may fit into the H_2O 'lattice' rather isomorphically, minimizing the disruption to the remaining water structures.

In the Rb-Z-DNA structure refinement, all the electron-density peaks in the solvent area were initially assigned as water molecules. After iterative refinement (positional and individual *B* factor) and adjustment of the water molecules according to the electron-density map, an *R* factor of around 0.30 was obtained. At this stage of refinement, some H_2O molecules had temperature factors of as low as 2.0 $Å^2$. This *B* value is the lowest value that the *X-PLOR* program will allow and is less than the average *B* factor of the DNA in the structure. These low *B* values indicate that there is a high electron density in the position, suggesting the atom should have more electrons than oxygen. After checking the locations and coordination of the sites, some of these electron-density peaks were assigned as Rb^+ ions. The *R* factor fell and was close to 0.20 after the assignment followed by refinement. After further cycles of positional refinement, *B*-factor refinement and simulated annealing, the *B* factors of some Rb^+ cations were more than 40 $Å^2$ when full occupancies were assigned to these Rb^+ ions, but were very low when the positions were assigned as water molecules. Accordingly, these positions were assigned as partially occupied Rb^+ sites. The water molecules around these Rb^+ ions were found to be disordered, with higher *B* factors and diffuse electron-density peaks at their locations. Partial occupancies and alternate structures were assigned for these disordered water positions. After further refinement, almost all Rb^+ ions had *B* factors below 30.0 $Å^2$ (Rb^+ 31 has a *B* factor of 31.8 $Å^2$). The final structure has three fully occupied Rb^+ sites and 13 Rb^+ sites with partial occupancies. A strong electron-density peak was found in a position located 3.09 Å from Rb^+ 88 and 2.90 Å from Rb^+ 41. Considering that RbCl was used in the crystallization and that Cl^- might be presented the crystal, a chloride ion was assigned to this electron-density peak. After refinement, the *B* factor for this fully occupied Cl^- site is 26.5 $Å^2$.

An electron-density map centered on Rb^+ 88 is shown in Fig. 2.

The coordination, occupancy and *B* factors of the Rb^+ ions assigned in the structure are listed in Table 2.

Most Rb^+ ions assigned in the structure have five or more coordination ligands and no regular coordination geometry. These rubidium positions coordinate to the O atom of the phosphate and the O and N atoms of the base groups or connect to these atoms through water bridges. Ten of the rubidium ions directly coordinate to at least one phosphate O atom, whereas only one coordinates to N7 of guanosine. Five rubidium

Table 1
Crystal data and refinement parameters.

(*a*) Data and parameters.

Unit-cell parameters (Å)	a = 17.92, b = 31.12, c = 43.96
Space group	$P2_12_12_1$
Max. resolution (Å)	1.70
Highest resolution shell (Å)	1.76–1.70
No. of reflections used	2537
$R_{\rm merge}$†	0.061
Redundancy	2.9
No. DNA atoms	376
No. Rb^+ ions	3 full, 13 partial
No. water molecules	67 full, 24 partial
No. chloride ions	1
Average *B* factors ($Å^2$)	
DNA	5.2
Waters	13.7
Final *R* factor	0.175

(*b*) Data-reduction statistics.

Resolution range (Å)	No. unique reflections	Completeness (%)	$R_{\rm square}$‡
30.00–3.66	321	93.9	0.102
3.66–2.91	289	91.2	0.062
2.91–2.54	282	94.3	0.059
2.54–2.31	282	93.4	0.072
2.31–2.14	281	95.6	0.063
2.14–2.02	270	94.7	0.068
2.02–1.91	275	94.5	0.068
1.91–1.83	264	95.0	0.093
1.83–1.76	273	93.2	0.074
1.76–1.70	18	6.1	0.096
All data	2555	85.3	0.082

† $R_{\rm merge} = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i \langle I(hkl)_i\rangle$. ‡ $R_{\rm square} = \sum[(I - \langle I\rangle)^2]/\sum I^2$.

Table 2
Coordination, occupancy and *B* factor of Rb^+ ions assigned in the Rb-Z-DNA structure.

[#] denotes a symmetry-related position. The coordinated chloride ion denoted as Cl is also listed.

Rb^+ residue number	Occupancy	*B* factor (Å^2)	Coordinated DNA atoms and distance (Å)	No. of coordinated H_2O/av. distance (Å)
32	1.00	13.6	G6O2P (3.19), G6O5′ (3.33), C9O1P[#] (3.17), C9O2P[#] (2.82)	5/2.94
88	1.00	17.9	G2N7 (2.99), C11O1P[#] (2.87), Cl (3.09)	5/3.04
111	1.00	21.8	C3O1P (2.79)	4/2.97
34	0.75	26.1	G2O3′ (3.10), C3O1P (2.83)	6/3.14
31	0.50	31.8	G6O6 (2.72), G12O6[#] (2.91)	6/2.92
40	0.50	23.8	None	8/3.07
41	0.50	23.8	G2O6 (2.71), Cl (2.90)	3/3.04
210	0.50	26.6	G2O2P (2.77)	8/3.0
211	0.50	21.9	G8O2P (2.74), C7O3′ (3.22)	6/2.99
212	0.50	20.4	C1O2 (2.60)	3/2.68
320	0.50	24.8	G8N2 (2.94), C9O2P (2.62)	3/2.82
321	0.50	23.8	C7O2 (2.80)	3/2.66
322	0.50	16.6	G2O1P (2.94)	6/3.06
323	0.50	27.7	G2O1P (2.93), C1O3′ (3.47)	6/2.99
379	0.50	26.4	G4O6 (2.90), G8O6 (2.97)	2/3.27
284	0.50	19.7	G10O2P (2.84), C9O3′ (3.32)	4/3.09

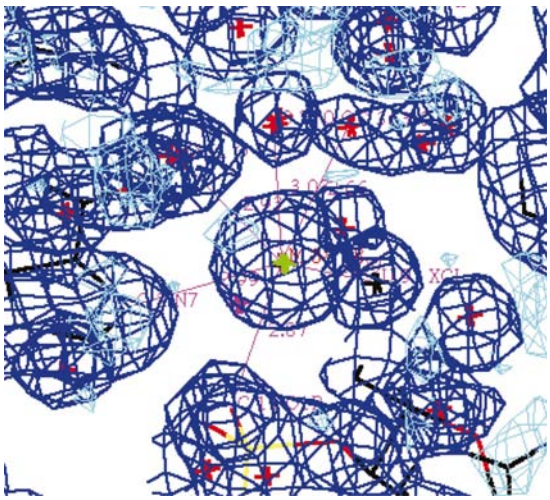

Figure 2
Electron-density map of the Rb-Z-DNA structure centered on Rb^+ 88. Rb^+ 88 is drawn as green star. Water molecules and a chloride ion are shown as red stars and a black star, respectively. The $2F_o - F_c$ map (blue net) and $F_o - F_c$ map (cyan net) were drawn at 2.0σ.

ions bind to the carbonyl O atoms of the bases. The distances between Rb^+ ions and the coordinated ligand varied from 2.6 to 3.3 Å. This result is similar to other published studies with near-atomic resolution data in which the distances between Rb^+ ions and ligand were found to be in the range 2.8–3.5 Å (Tereshko *et al.*, 1999, 2001).

Rubidium ions assigned in the structure have the function of stabilizing the DNA structure by cross-linking two strands or two duplexes of the DNA. Rb^+ 32 and Rb^+ 88 bind both the primary and symmetry-related DNA, stabilizing the lattice. Rb^+ 31 bridges two DNA duplexes which are stacked on each other. Rb^+ 379 connects the O6 of guanosine 4 and the O6 of guanosine 8, bridging two strands in one DNA duplex. Rb^+ 41 coordinates to the O6 of guanosine 2. It also connects two strands in one duplex and a symmetry-related molecule through a water bridge. Rb^+ 40 connects two strands in one duplex through a water bridge. A group of strong electron-density peaks is found in the electronegative pocket of the crystal lattice formed by the phosphate O atoms and the base N atoms of C7, G8 and C9 and the phosphate groups of symmetry-related molecules. These electron-density peaks appeared to be too strong to be considered as H_2O but too close to be assigned as simultaneously occupied Rb^+ sites. Rb^+ positions with partial occupancies were assigned to these electron-density peaks. Alternate structures were used for the Rb^+ ions in this group (Rb^+ sites numbered with 200s and 300s are in alternate structures). That is, when an ion is in one position, another position in an alternate structure is no longer favourable for the Rb^+ ion. For the partially occupied positions, half occupancies were used in the structure (except Rb^+ 34, with an occupancy of 0.75). With the concern about the accuracy of the result, no population refinement was performed in this work. The relatively large number of partially occupied sites suggested that there are numerous relatively low energy sites for binding of monovalent cations to Z-DNA and many of them are cation–water hybrid positions.

The results of this work indicate that in the Z-DNA structure most of the monovalent cations are mobile. The mobility of the cations depends on the position of the ions in the DNA crystal and the temperature environment. The cations exchange with water molecules and have fractional occupancies, which makes it difficult to make an unambiguous Rb^+ position assignment. This result agrees with the conclusion of Matthew Young's molecular-dynamic simulations, which suggested that the structures of monovalent counterion in DNA are not static but dynamic (Young *et al.*, 1997). Studies using anomalous diffraction to identify the position of the Rb^+ ions in the Z-DNA structure will be the subject of further research.

I thank Professor Gary Quigley for his academic guidance and great help in the research. I am very grateful to Dr Wladek Minor for his help with the *DENZO* program and Dr Shun-le Chen for his assistance in using computer software.

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