

Amine free crystal structure: The crystal structure of d(CGCGCG)₂ and methylamine complex crystal

Hirofumi Ohishi^{a,*}, Koji Tsukamoto^b, Yoichi Hiyama^c, Naoyoshi Maezaki^b,
Tetsuaki Tanaka^b, Toshimasa Ishida^a

^a Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan

^b Graduated School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

^c Genomic Science Laboratories Group III, Dainippon Sumitomo Pharmaceutical Co., Ltd., 3-1-98 Kasugadenaka, Knochana-ku, Osaka, Osaka 554-0022, Japan

Received 25 June 2006

Available online 13 July 2006

Abstract

We succeeded in the crystallization of d(CGCGCG)₂ and methylamine Complex. The crystal was clear and of sufficient size to collect the X-ray crystallographic data up to 1.0 Å resolution using synchrotron radiation. As a result of X-ray crystallographic analysis of 2F_o – F_c map was much clear and easily traced. It is the first time monoamine co-crystallizes with d(CGCGCG)₂. However, methylamine was not found from the complex crystal of d(CGCGCG)₂ and methylamine. Five Mg ions were found around d(CGCGCG)₂ molecules. These Mg ions neutralized the anion of 10 values of the phosphate group of DNA with five Mg²⁺. DNA stabilized only by a metallic ion and there is no example of analyzing the X-ray crystal structure like this. Mg ion stabilizes the conformation of Z-DNA. To use monoamine for crystallization of DNA, we found that we can get only d(CGCGCG)₂ and Mg cation crystal. Only Mg cation can stabilize the conformation of Z-DNA. The method of using the monoamine for the crystallization of DNA can be applied to the crystallization of DNA of long chain of length in the future like this.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Z-DNA; X-ray crystallography; Crystal structure; Methylamine

Up to now, a lot of X-ray crystallographic structure analyses of DNA have been done [1]. A metallic ion and polyamine are used for the crystallization in all examples of the X-ray crystal structure analysis of DNA [2]. It is usual that a metallic ion groove-bound to the minor groove of DNA and polyamine forms the bridged structure coordinated to the guanine base and phosphoric acid between DNA that could move by the symmetry in the crystal structure [3]. On the other hand, metallic ions are used to neutralize the anion of the phosphoric acid of DNA generally and to change B type DNA to Z type DNA in conformation [4,5]. Polyamine connects with the minor groove of DNA, is used to stabilize DNA [6,7], and is used to form the bridged structure between some DNA and to

stabilize the structure of DNA. The position of spermine and a metallic ion is not clarified in low resolution at that time though it is done by the X-ray crystal structure analysis and clarified those structures in 1982 A type DNA [8], in 1980 B type DNA [9], and in 1979 Z type DNA [10]. The X-ray crystal structure analysis of the high-resolution evaluation becomes possible by using the synchrotron radiation now and the complex structure of various polyamines, metallic ion, and DNA is clarified. As for a certain kind of polyamine, it is suggested that there were correlations between the cell proliferation [11] and the amount of polyamine because quite a lot of spermidine exists in the cancer cell [12] and the maturity rat [13]. On the other hand, a lot of data have not been made public for a metallic ion. The d(CGCGCG)₂ took left-handed Z type structure with double helices in the complex crystal of spermidine [14], PA(24) [15] of three value, of the charge

* Corresponding author. Fax: +81 72 690 1090.

E-mail address: ohishi@gly.oups.ac.jp (H. Ohishi).

number. This DNA molecule was connected with one spermidine and one PA(24) molecule, respectively, and three pieces of Mg^{2+} and one Na^+ and N7th position of the guanine base and phosphoric acid were directly connected to the metal cations. The $d(CGCGCG)_2$ took left-handed Z type structure in the complex crystal with spermine [16], thermospermine [17], and PA(222) [7] of four values of the charge number. Each spermine, thermospermine, and the PA(222) molecule connected by two molecules and one Mg^{2+} connected with this DNA molecule. It is clarified as regards polyamine that it does not bind with DNA when the permittivity of the polyamine is low and a metallic ion binds with DNA by priority. We were able to obtain the data of 1.0 Å resolution by making the complex crystal with $d(CGCGCG)_2$ and CH_3NH_3 (methylamine) which is monoamine this time using the synchrotron radiation. The X-ray crystal structure analysis was described in this thesis. The crystal structure did not contain the amine and only five Mg^{2+} connected though this DNA also took

left-handed Z type structure with double helices as well as another. This crystal structure consists only of DNA and a metallic ion, and there is no such example from the analysis conducted so far. This crystal structure might give some suggestions as to the stabilization of DNA.

Experiment

DNA $d(CGCGCG)_2$ was synthesized by Takara BIO INC. and it was offered. Methylamine was bought from Wako Pure Chemical Industries, Ltd. 4 mg/ml DNA $d(CGCGCG)_2$, 100 mM $MgCl_2$, and 30 mM methylamine of DNA were adjusted in 10 mM Cacodylic acid buffer, pH 7.0, and the MPD of the liquid was adjusted at 7%, and the MPD of the outside liquid was adjusted by 10–15% and the crystallization solution was left sitting for 2 weeks by the vapor diffusion equilibrium method in the incubator at 20 °C. A pillar-shaped transparent colorlessness of $0.4 \times 0.4 \times 0.2$ mm crystal was obtained 2 weeks later. Data were measured by the synchrotron radiation by using beamline BL-6A of High Energy Accelerator Research Organization. The data measured the wavelength of the synchrotron radiation of 1.0 Å with the ADSC Quantum-210 CCD camera in data up to 1.0 Å resolution under the Crayo condition of 95 °K. The data were measured of 120 frames at Φ angle from -90° to 90° by 1.5 degrees as for data at exposure time 2 s. Data processing obtained 98,000 reflections by using program mosflm [18]. 11,000 independent reflections up to 1.0 Å were able to be obtained with scalea and truncate of CCP4 [19]. Completeness at this time was 99.9% and *R*-merge was 0.053. Rotation Translation function was calculated with program Amore by using these data and the phase was decided. Coordinates of DNA of PA(24) [15] were used for the phase decision. The position of Mg^{2+} and the position of water were able to be decided with Turbo Frodo [20] by using the difference Fourier synthesis map of $2F_o - F_c$ [21]. The structure was refined with the DNA which was the anisotropic temperature factor and Mg^{2+} and the water molecule were in the isotropic temperature factor by programs X-PLOR [22], and SHELXL [23], and final *R* factor value was 0.14. Data collecting statistics for $d(CGCGCG)_2$ + methylamine are given in Table 1. The atomic coordinates have been deposited in the Brookhaven Protein Databank (PDB entry number 2HYH).

Table 1
The crystal data of $d(CG)_3$ -methylamine complex

Cell dimensions	Methylamine complex
<i>a</i> (Å)	17.88
<i>b</i> (Å)	30.84
<i>c</i> (Å)	43.96
α (°)	90
β (°)	90
γ (°)	90
<i>V</i> (Å ³)	24,244
Space group	P2 ₁ 2 ₁ 2 ₁
Crystal system	Orthorhombic
No. of reflections measured	98,000
No. of reflections independent	10,340
No. of reflections used 3σ	10,136
Completeness	100
Used reflections completeness	98.0
Resolution (Å)	1.0
<i>Z</i>	4
<i>R</i> -value	0.139

Result and discussion

It was the same space group and crystal system of the other $d(CGCGCG)_2$ + polyamine complex crystal and the crystal of $d(CGCGCG)_2$ + methylamine complex was

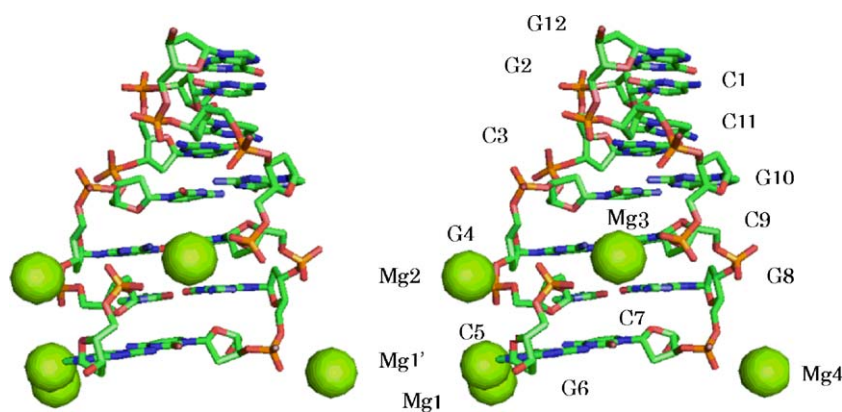


Fig. 1. The stereoscopic structure of amine free $d(CGCGCG)_2$ and methylamine complex. Red sticks show oxygen atoms, orange sticks show phosphorus atoms, blue sticks show nitrogen atoms, and green sticks show carbon atoms and right green balls show magnesium cations. DNA forms left-handed Z-DNA. Five magnesium cations bind to DNA through water molecule or directly coordinated to N7th position of guanine base. Black characters show numbering system of the nucleic acid base and the number of the magnesium ion. G is guanine and C is cytosine in the nucleic acid base.

Download English Version:

<https://daneshyari.com/en/article/1939217>

Download Persian Version:

<https://daneshyari.com/article/1939217>

[Daneshyari.com](https://daneshyari.com)