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Polyamines stabilize left-handed Z-DNA: Using X-ray crystallographic analysis, we have found a new type of polyamine (PA) that stabilizes left-handed Z-DNA

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Abstract

There are many great reports of polyamine stabilization of the Z-DNA by bridge conformation between neighboring, symmetry-related Z-DNA in the packing of crystals. However, polyamine binding to the minor groove of Z-DNA and stabilizing the Z-DNA structure has been rarely reported. We proved that the synthesized polyamines bind to the minor groove of Z-DNA and stabilize the conformation under various conditions, by X-ray crystallographic study. These polyamines consist of a polyamine nano wire structure. The modes of the polyamine interaction were changed under different conditions. It is the first example that the crystals consisted of metal free structure. This finding provides a basis for clarifying B–Z transition mechanics.

Keywords: Polyamine; Z-DNA; B–Z transition; X-ray crystallography; *Ab-initio* calculation

Until now the base pairs found by Watson and Crick [1] and the B-DNA found by Dickerson and Drew [2] have been believed to be genetic. In addition, the A-DNA discovered by Sundararingan collected as gene in which the handedness of the gene differed from B-DNA. It is the left-handed Z-DNA found by Rich which overthrew the established knowledge that genes are only right-handed, Z-DNA existence *in vivo* actually was not made clear, though the discovery of Z-DNA itself was shocking. The detection of the left-handed Z-form of DNA in 1979 by Rich [3] caused a large upheaval in studies of the chemistry of the gene. The role of the Z-form was not immediately evident. It has been obvious that B-DNA changes to the left-handed Z-DNA form under a high salt concentration or a very high concentration of polyamine solution [4,5]. The B–Z transition mechanism has not been established, though molecular dynamics calculations which utilize computation in order to clarify the mechanisms of the B–Z transition [6–8], and various solution experiments,

Abbreviations: PA(24), N1-(2-amino-ethyl)-butane-1,4-diamine; PA(222), N1-[2-(2-amino-ethylamino)-ethyl]-ethane-1,2-diamine; PA(2222), N1-{2-[2-(2-amino-ethylamino)-ethylamino]-ethyl}-ethane-1,2-diamine; ADAR1, adenosine deaminase reductase.

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etc. have been carried out [9,10]. A considerable advance emerged with the discovery of the ADAR1 protein [11], because X-ray crystallographic analysis was performed on the ADAR1 protein and Z-DNA complex crystal by Rich at 1999 [12]. It was further clarified that left-handed Z-DNA existed for 98 of 137 human genes examined [13]. It was then discovered that the supercoil of the vector relaxes by Z-DNA, and that as the supercoil of the topoisomerase relaxes naturally it is helped, and Z-DNA shoulders the transcriptional enhancement of DNA by these effects [14]. In addition, the ADAR1 protein was also manifestly a cause of the error coding on the emergence of Z-DNA, since it is the enzyme which converts the adenine into guanine [15]. However, the role of the polyamine and stabilization mechanism of Z-DNA have not yet been proven at present. No one has succeeded in the crystallization of long stem Z-DNA and a combination of B-DNA and Z-DNA. If the structure of long stem Z-DNA and the combination of B-DNA and Z-DNA became clear, the B–Z transition and transcription mechanism could also be revealed. Therefore, elucidation of the stabilization of Z-DNA is essential. We endeavored to clarify the roles of the polyamines on the stabilization mechanism of Z-DNA and performed crystallographic structure analysis of the complex crystal of Z-DNA with six kinds of polyamines. To the best of our knowledge, this is the first instance that a polyamine stabilizing Z-DNA has been synthesized. Structure of the Z-DNA and polyamine complex, just like a left-handed triplex, was taken such that the polyamine combined in the minor groove of Z-DNA. At present, right-handed cross-links and a triplex are the most common structures [16], so this triplex-like left-handed Z-DNA structure can be constructed to be rare. These structures of polyamines and Z-DNA depended on the thermodynamic conditions around the Z-DNA. This is the first evidence that the total energy of the Z-DNA and polyamine complex is related to enthalpy in these crystals. A relationship between the crystal structure and thermodynamic conditions is described in this manuscript. The interaction with the ADAR1 protein and the role of Z-DNA in vector DNA will be clarified in future work.

Results and discussion

It is very interesting that natural polyamines possess only a propyl or butyl group. We synthesized a polyamine with an ethyl unit. It is an odd feature that while the ethylamino unit is combined in the minor groove of left-handed Z-DNA at ordinary-temperatures, the propylamino and butylamino units, which exist naturally, are not combined in the minor groove of left-handed Z-DNA at ordinary-temperature. The phenomenon is utilized in forming the cross-link between symmetry-related neighboring Z-DNA. Three kinds of natural polyamine have formed the cross-link between symmetry-related adjoining Z-DNA without combining in the minor groove of Z-DNA. The Z-DNA announced by Rich et al. formed a complex with spermine at the minor groove under conditions of cooling to −110° [17]. At very low temperatures, however, spermine is a natural polyamine. The $d(CG)_3 + PA(24)$ complex in this study is the first example of an ordinary-temperature polyamine combined in the minor groove [18]. Therefore, we synthesized PA(222) as a result of X-ray crystal structure analysis of $d(CG)_3$ and the PA(222) complex, with one PA(222) molecule perfectly combining in the minor groove of left-handed Z-DNA [19], and it stabilized Z-DNA. It is necessary to determine why PA(222) and the ethylamino unit recognize the Z-DNA minor groove. Towards this end we minutely examined the complex molecular structure of PA(24), PA(222) and Z-DNA, and a natural polyamine and Z-DNA. As the result, it was made clear that the ethylamino unit was just equal to the distance between the bases and phosphoric acids of the Z-DNA. It was apparent that the propyl and butylamino acid unit exceeded the length needed in order to combine the bases and the phosphoric acids. However, water goes into the minor groove, since enthalpy decreases at low temperatures, and the combination of the propylamino unit and the butylamino unit in the minor groove through the water that is present becomes possible. In order to approach the *in vivo* conditions, the last PA(2222) molecule was synthesized which was longer than PA(222) molecule at the ethylamino unit. We tried to crystallize this PA(2222) with $d(CG)_3$ under three different conditions. As expected, the property of becoming crystals worked very well under the expected conditions. Very stable crystals were obtained by examining a large number of crystallization conditions. The electron densities were very clearly traced by the polyamines. The electron densities of two PA(2222) molecules at room temperature, at low temperature, and under high salt conditions are shown in Supplementary Figs. 4-1a, 4-1b, 4-2a, 4-2b, and 4-3a, 4-3b, respectively. The electron density of a base pair was shown in Supplementary Fig. 4-4. In the case of the $PA(222) + d(CG)_3$ complex, one PA(222) molecule combines for each double stranded Z-DNA in the minor groove, and another PA(222) molecule formed a cross-linked structure with symmetry-related adjoining Z-DNA. However, in spite of the chain length of the PA(2222) molecule longer, two PA(2222) molecules combined in the minor groove of Z-DNA, as shown in Fig. 1A, and it assumed the very rare structure of a left-handed triplex, as shown in Fig. 2A. Therefore, the PA(2222) molecule did not locate between symmetry-related neighboring Z-DNA. One PA(2222) molecule connected to the minor groove of the recess of Z-DNA, therefore an amino and imino group of PA(2222) bind to the base group of an O2 atom of cytidine and an N2 atom of the guanine group of Z-DNA. Another PA(2222) molecule bound to the oxygen of a phosphate group in the minor groove of Z-DNA as shown in Fig. 1A. This is the first evidence that two polyamine molecules can bind to the minor groove of Z-DNA. In addition, in all the polyamine and Z-DNA complex crystals except for the PA(2222) complex, although