Crystal Structure of a Junction between B-DNA and Z-DNA

Z-DNA is a higher-energy form of double stranded DNA (dsDNA) with left-handed helical sense whereas most DNA is found as energetically stable B-DNA with right-handed helical sense. Z-DNA is stabilized by high salt or in the presence of positively charged molecules such as spermine *in vitro*. However, negative supercoiling is thought to be a Z-DNA stabilizing factor *in vivo*. It has been shown that negative supercoiling induced by transcription stabilizes the Z conformation behind the RNA polymerase. Several studies have suggested that the Z conformation is not only a result of transcription, but can act as a *cis*-element regulating the transcriptional status of a gene. Moreover, the identification of a family of polypeptides containing a domain specific for Z-DNA binding has supported the involvement of Z-DNA in biological processes as yet unidentified [1].

The identification of Z-DNA binding proteins and the biological evidences supporting the presence of Z-DNA *in vitro* have raised questions concerning the B-Z junction, the region linking right-handed B-DNA and left-handed Z-DNA. When a region is converted to Z-DNA within a long continuous double stranded B-DNA, two B-Z junctions will be formed flanking the region of Z-DNA. Many studies have been carried out to characterize the B-Z junction using biochemical and biophysical methods, however, the structural properties of B-Z junction have not been well studied. It is difficult to prepare a continuous hybrid B/Z DNA duplex containing both B and Z conformations in physiological conditions, which is necessary for structural analysis at high resolution. In this study, we have taken advantage of the binding of $hZ\alpha_{ADAR1}$, a Z-DNA binding domain from human ADAR1 [2], in order to stabilize the Z conformation in one-half of the DNA duplex. We designed a 15-base pair dsDNA consisting of two regions: one is CG rich and can be easily converted to Z-DNA in Z-DNA inducing conditions, and the other region is TA rich and maintains B conformation predominantly (Fig. 1). Therefore, in the presence of the $hZ\alpha_{ADAR1}$, one B-Z junction is thus formed in the middle of the DNA duplex, connecting the Z- and B-DNA. The $hZ\alpha_{ADAR1}$ domain (aa.140-202) was co-crystallized with the DNA [5'-GTCGCGCGCCATAAACC-3' and 5'-ACGGTTTATGGCGCGCG-3']. The crystal has the space group *P*61, and the structure of the complex was solved at 2.6 Å resolution using the data obtained at beamline **BL41XU** of SPring-8 (Harima, Japan) and beamline BL-6B of PLS (Pohang, Korea) (R = 23.8 %, R_{free} = 28.5 %; ref. [3]).

Overall structure of the dsDNA is composed of eight base-pair Z-DNA, six base-pair B-DNA and the B-Z junction (Figs. 1, 2). The Z-DNA is stabilized by four Z-DNA binding domains ($hZ\alpha_{ADAR1}$, Fig. 2) and shows standard base-pair step parameters of Z-DNA (Fig. 2 and Table 1). B-DNA also has standard base-pair step parameters of B-DNA (Fig. 2 and Table 1). At the B-Z junction, A and T bases are extruded from the duplex, thereby linking left-handed Z-DNA to right-

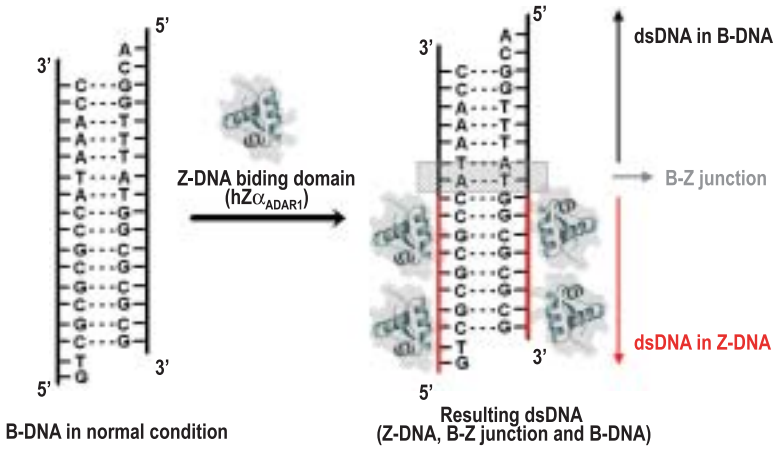

Fig. 1. The strategy of making a stable junction between B-DNA and Z-DNA. Four Z-DNA binding proteins from human ADAR1 ($hZ\alpha_{ADAR1}$) stabilize the Z-conformation in one-half of a 15 base pair dsDNA and other region remains as a junction and B-DNA.

Table 1 The base pair parameters near the junction between B-DNA and Z-DNA and the standard B- and Z- DNAs

Base step	Shift	Slide	Rise	Tilt	Roll	Twist
A3/A2	0.26	-0.20	3.49	1.82	-3.02	38.51
A2/T1	-0.26	1.46	3.37	-4.56	6.82	38.57
C-1/C-2	0.28	5.83	-4.60	8.23	4.48	-12.96
C-2/G-3	0.28	-1.34	-3.09	1.50	-8.14	-47.51
B-DNA	-0.02	0.12	3.36	-0.19	0.02	35.58
Z-DNA	-0.03	2.81	3.49	0.32	-3.14	-25.10

Each parameter was calculated using the program 3DNA. Parameters of B-DNA were calculated using crystal structure of B-DNA[4] (PDB ID: 1BNA), and those of Z-DNA were calculated using the crystal structure of Z-DNA[3] (PDB ID: 2DCG)

handed B-DNA. The base-pair step parameters of B-Z junction have 3.82 Å rise (Dz) and -16.9º twist (Fig. 2 and Table 1). Though one junctional base is extruded, base stacking is continuous from Z-DNA to B-DNA through the B-Z junction without significant impairment. Thus, base stacking is also a major stabilizing factor even at the B-Z junction as well as in B-DNA and Z-DNA regions (Fig. 2, [3-5]). It seems that extrusion of a base from each strand accommodates a reversal in the helical direction of the backbone from B- to Z conformation. It is also speculated that these extruded bases may be specific recognition sites for enzymes mediating DNA modification.

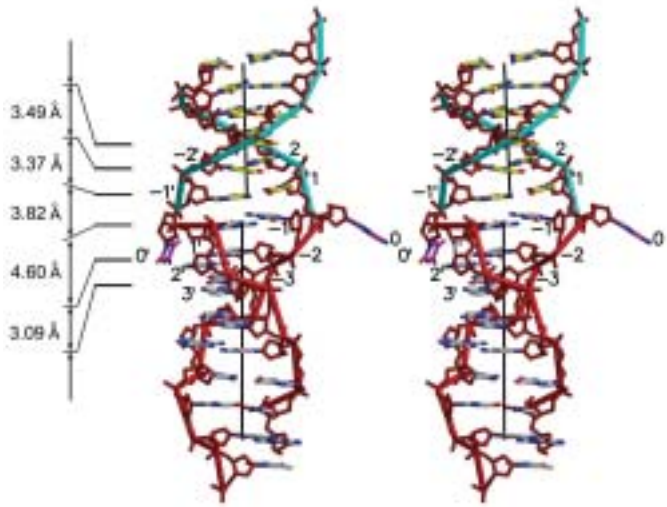

Fig. 2. Skeletal stereo views of DNA. Red and blue lines connect phosphate groups in Z-DNA and B-DNA, respectively. One strand has numbers with a prime symbol. Negative numbers start at the 5‘ end and increase toward the extruded junctional bases A0 (right) and T0′(left). The rises of base step parameters are shown.

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