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NMR study of hydrogen exchange during the B–Z transition of a DNA duplex induced by the Zα domains of yatapoxvirus E3L

Eun-Hae Lee [a,1], Yeo-Jin Seo [a,1], Hee-Chul Ahn [b], Young-Min Kang [a], Hee-Eun Kim [a], Yeon-Mi Lee [a], Byong-Seok Choi [c,*], Joon-Hwa Lee [a,*]

[a] Department of Chemistry, and RINS, Gyeongsang National University, Jinju, Gyengnam 660-701, Republic of Korea
[b] Advanced Analysis Center, KIST, Seoul 130-650, Republic of Korea
[c] Department of Chemistry, KAIST, Daejeon 305-701, Republic of Korea

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ABSTRACT

The Yaba-like disease viruses (YLDV) are members of the Yatapoxvirus family and have double-stranded DNA genomes. The E3L protein, which is essential for pathogenesis in the vaccinia virus, consists of two domains: an N-terminal Z-DNA binding domain and a C-terminal RNA binding domain. The crystal structure of the E3L orthologue of YLDV (yabZα$_{E3L}$) bound to Z-DNA revealed that the overall structure of yabZα$_{E3L}$ and its interaction with Z-DNA are very similar to those of hZα$_{ADAR1}$. Here we have performed NMR hydrogen exchange experiments on the complexes between yabZα$_{E3L}$ and d(CGCGCG)$_2$ with a variety of protein-to-DNA molar ratios. This study revealed that yabZα$_{E3L}$ could efficiently change the B-form helix of the d(CGCGCG)$_2$ to left-handed Z-DNA via the *active-mono* B–Z transition pathway like hZα$_{ADAR1}$.

1. Introduction

The Yaba monkey tumor virus (YMTV) and Yaba-like disease virus (YLDV) are members of the Yatapoxvirus family and have double-stranded DNA genomes [1]. All poxviruses have a gene called E3L that is essential for pathogenesis in the vaccinia virus [2–5]. The E3L protein consists of two domains: an N-terminal Z-DNA binding domain and a C-terminal RNA binding domain [2,3,6,7]. This N-terminal region shows sequence homology to the Zα domains found in human ADAR1 (hZα$_{ADAR1}$) [8] and in the IFN-inducible DLM-1 of mammals [9] (see Fig. 1A). The Z-DNA binding affinity of the Zα domain of E3L is required for viral pathogenicity [2].

The crystal structure of the Zα domain of the YLDV E3L orthologue (yabZα$_{E3L}$) bound to Z-DNA revealed that the monomeric Zα domain binds to one strand of Z-DNA while a second monomer binds to the opposite strand, yielding twofold symmetry with respect to the helical axis [4]. The overall structure of yabZα$_{E3L}$ and its interaction with Z-DNA are very similar to those of hZα$_{ADAR1}$ and mZα$_{DLM1}$ [8,9]. Recent NMR studies of the d(CGCGCG)$_2$–hZα$_{ADAR1}$ complex [10] have suggested the *active* B–Z transition mechanism of a six-base-paired (6-bp) DNA duplex (Fig. 1B), in which the hZα$_{ADAR1}$ protein first binds to B-DNA and then converts it to left-handed Z-DNA, a conformation that is then stabilized by the additional binding of a second hZα$_{ADAR1}$ molecule.

Here, to investigate the molecular mechanism of the B–Z transition of a DNA duplex induced by the yabZα$_{E3L}$, we have performed NMR hydrogen exchange experiments on the complexes formed by yabZα$_{E3L}$ and d(CGCGCG)$_2$ (referred to as CG6, Fig. 2B) with a variety of protein-to-DNA (P/N) molar ratios. Comparison of these results with those from the analysis of hZα$_{ADAR1}$–CG6 in a previous study [10] leads to valuable insights into the molecular mechanism of the B–Z transition of a DNA duplex induced by the yabZα$_{E3L}$ protein.

2. Materials and methods

2.1. Sample preparation

The DNA oligomer d(CGCGCG) was purchased from M-biotech Inc. (Seoul, Korea). The DNA oligomer was purified by a reverse-phase

* Corresponding authors. Fax: +82 42 350 8120 (B.-S. Choi); +82 55 761 0244 (J.-H. Lee).
E-mail addresses: byongseok.choi@kaist.ac.kr (B.-S. Choi), joonhwa@gnu.ac.kr (J.-H. Lee).
[1] These authors contributed equally to this work.

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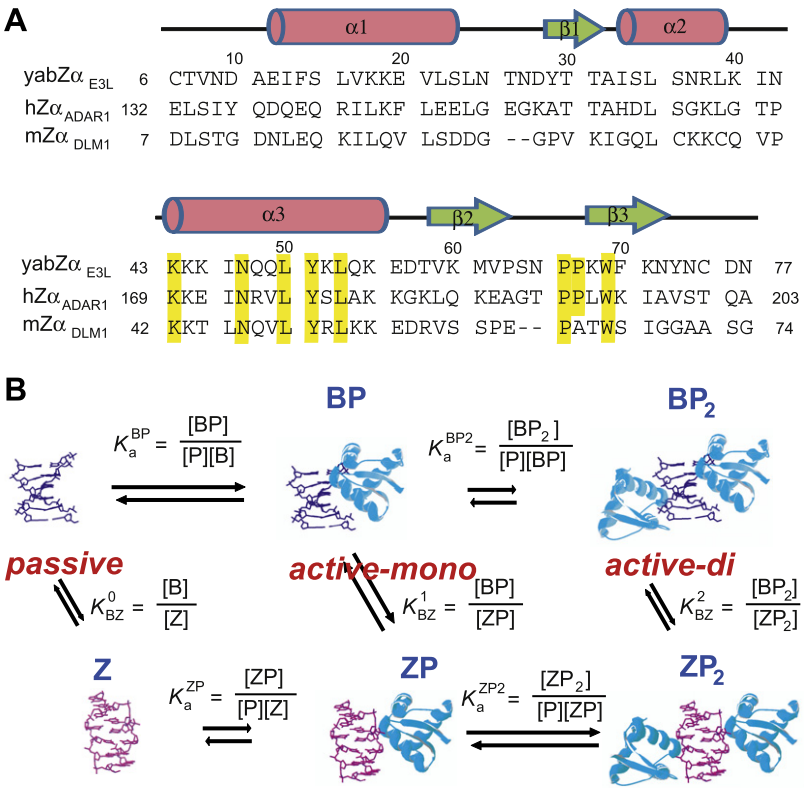

Fig. 1. (A) Multiple sequence alignment of the Zα proteins. Numbering and secondary structure elements for the Zα domains of E3L from YLDV (yabZα$_{E3L}$), human ADAR1 (hZα$_{ADAR1}$), and mouse DLM1 (mZα$_{DLM1}$). Secondary structure is drawn on top of the sequence. (B) B–Z transition of a 6-bp DNA duplex by two Z-DNA binding proteins. B and Z indicate the B-form and Z-form of the DNA duplex and P indicates the Z-DNA binding proteins.

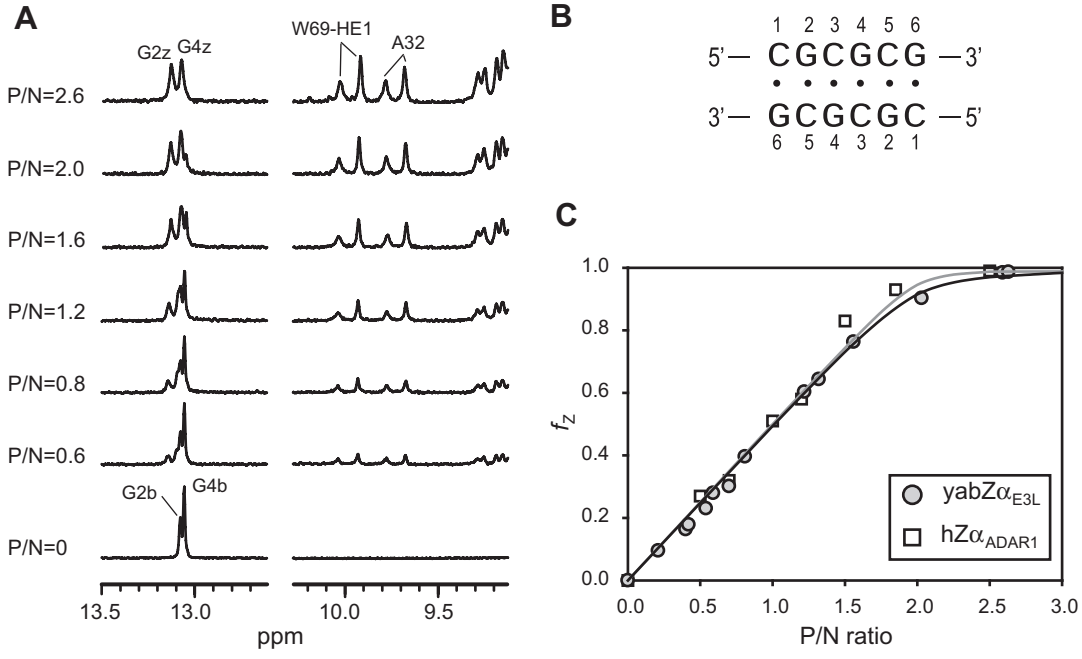

Fig. 2. (A) The 1D proton spectra of the CG6 DNA duplex in a 90% H_2O/10% D_2O solution containing 10 mM sodium phosphate (pH = 8.0) and 100 mM NaCl at 35 °C upon titration with yabZα$_{E3L}$. The P/N ratios are shown on the left of each spectrum. (B) Sequence contexts of the CG6 DNA duplex. (C) The fraction of Z-DNA (f_Z) of the CG6 induced by yabZα$_{E3L}$ (gray circle) and hZα$_{ADAR1}$ (open square) [10] at 35 °C as a function of the P/N ratio. Solid lines are simulated f_Z of the DNA duplexes induced by yabZα$_{E3L}$ (black) and Zα$_{ADAR1}$ (gray).

HPLC and desalted using a Sephadex G-25 gel filtration column. The amount of the DNA duplex [d(CGCGCG)$_2$, CG6] was represented as the concentration of double-stranded sample. To produce ^{15}N-labelled or ^{13}C, ^{15}N-labeled yabZα$_{E3L}$, BL21(DE3) bacteria were grown in M9 medium containing 1 g/L $^{15}NH_4Cl$ and/or 2 g/L ^{13}C-glucose. Expression and purification of ^{15}N-labelled or ^{13}C, ^{15}N-labeled