Biochemical and Biophysical Research Communications 482 (2017) 335–340

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

NMR elucidation of reduced B-Z transition activity of PKZ protein kinase at high NaCl concentration

Ae-Ree Lee [a], Yeo-Jin Seo [a], Seo-Ree Choi [a], Kyoung-Seok Ryu [b], Hae-Kap Cheong [b], Shim Sung Lee [a], Masato Katahira [c], Chin-Ju Park [d, *], Joon-Hwa Lee [a, b, **]

[a] Department of Chemistry and Research Institute of Natural Science, Gyeongsang National University, Gyeongnam 52828, Republic of Korea
[b] Division of Magnetic Resonance, KBSI, Chungbuk 28119, Republic of Korea
[c] Institute of Advanced Energy, Kyoto University, Kyoto 611-0011, Japan
[d] Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea

ARTICLE INFO

Article history:
Received 10 November 2016
Accepted 11 November 2016
Available online 14 November 2016

Keywords:
NMR
Z-DNA
Salt effect
Z-DNA binding protein
DNA-protein interaction

ABSTRACT

A Z-DNA binding protein (ZBP)-containing protein kinase (PKZ) in fish species has an important role in the innate immune response. Previous structural studies of the Zα domain of the PKZ from *Carassius auratus* (caZα$_{PKZ}$) showed that the protein initially binds to B-DNA and induces B-Z transition of double stranded DNA in a salt concentration-dependent manner. However, the significantly reduced B-Z transition activity of caZα$_{PKZ}$ at high salt concentration was not fully understood. In this study, we present the binding affinity of the protein for B-DNA and Z-DNA and characterize its extremely low B-Z transition activity at 250 mM NaCl. Our results emphasize that the B-DNA-bound form of caZα$_{PKZ}$ can be used as molecular ruler to measure the degree of B-Z transition.

1. Introduction

Z-DNA binding proteins (ZBPs) are critical players in various cellular functions such as RNA editing and the innate immune response [1–3]. An RNA editing enzyme (ADAR1), DNA-dependent activator of interferon-regulatory factor (DAI), the viral E3L protein, and a fish protein kinase containing a ZBP (PKZ) are examples of ZBPs which contain one or more Z-DNA binding domains [1,2,4,5]. They stabilize left-handed Z-DNA, which is a higher energy conformation than B-DNA, via complex formation [6,7]. The conserved Zα domain (~60 amino acids) consists of three α helices and three β strands (α1-β1-α2-α3-β2-β3), the first half of which is considered as a helix-turn-helix fold (Fig. 1A) [8]. Several crystal structures of the Zα domain in complex with dT(CG)$_3$ revealed that two Zα molecules bind to each strand of double-stranded Z-DNA using residues in their α3 helix and their β-hairpin (β2-loop-β3) (Fig. 1B) [1,7,9,10]. The critical residues for Z-DNA binding are well conserved among various ZBPs [2]. In order to explain ZBP-DNA interaction, an active B-Z transition mechanism has been suggested (Fig. 1B). Firstly, the ZBP (denoted as **P**) binds to B-DNA (denoted as **B**) to form **BP**, and then **BP** is converted to **ZP** (Z-DNA is denoted as **Z**). Binding of another **P** to **ZP** generates a stable **ZP$_2$** complex [11].

In a previous study, we applied the active B-Z transition mechanism to the function of the Zα domain of the PKZ from *Carassius auratus* (caZα$_{PKZ}$) [12]. The solution structure of the free form of caZα$_{PKZ}$ was mostly similar to its structure when bound to Z-form dT(CG)$_3$, with the exception of the orientation of the β-hairpin, which is involved in a charge-charge interaction with the phosphate backbone of the Z-DNA [12]. However, the global analysis of chemical shift perturbation data at 10 mM and 100 mM NaCl indicated that the protein had different binding affinities for B- and Z-DNA and that interaction with B-DNA was severely affected by the concentration of NaCl ([NaCl]). By monitoring ellipticity at 255 nm for the B-Z transition with time course CD spectroscopy at various [NaCl], it has been shown that the B-Z transition activity of caZα$_{PKZ}$ is strongly salt concentration-dependent, unlike other ZBPs, and that high salt concentrations (tested up to 250 mM NaCl) produce extremely low activity [10].

In order to investigate the B-Z transition induced by caZα$_{PKZ}$ at high [NaCl], we analyzed the imino proton spectra of dT(CG)$_3$

Abbreviations: caZα$_{PKZ}$, the Zα domain of *Carassius auratus* protein kinase Z; ZBP, Z-DNA binding protein.

* Corresponding author.
** Corresponding author. Department of Chemistry and RINS, Gyeongsang National University, Gyeongnam 52828, Republic of Korea.
E-mail addresses: cjpark@gist.ac.kr (C.-J. Park), joonhwa@gnu.ac.kr (J.-H. Lee).

http://dx.doi.org/10.1016/j.bbrc.2016.11.064
0006-291X/

during titration with caZα_{PKZ} and monitored the ^{1}H-^{15}N chemical shift perturbations of caZα_{PKZ} during titration with dT(CG)$_3$, both at 250 mM NaCl. Based on these data, the relative Z-DNA population, the dissociation constants of caZα_{PKZ} for B- and Z-DNA binding, and the equilibrium constant for the B-Z transition were determined. Our study also provides information about the chemical shift changes in the B-DNA-bound form of caZα_{PKZ}, which can be used as a molecular ruler for the B-Z transition induced by ZBPs at 250 mM NaCl.

2. Materials and methods

2.1. Sample preparation

The DNA oligomer dT(CG)$_3$ was purchased from M-biotech Inc. (the Korean branch of IDT Inc.), purified by reverse-phase HPLC, and desalted using a Sephadex G-25 gel filtration column. To produce ^{15}N-labeled caZα_{PKZ}, BL21(DE3) bacteria expressing caZα_{PKZ} were grown in M9 medium containing 1 g/L of $^{15}NH_4Cl$. The expression and purification of ^{15}N-labeled caZα_{PKZ} have been described in a previous report [12]. The DNA and protein samples were dissolved in a 90% H_2O/10% D_2O NMR buffer containing 10 mM sodium phosphate (pH 6.0) and 250 mM NaCl.

2.2. NMR experiments

All NMR experiments were performed on an Agilent DD2 700 NMR MHz spectrometer (GNU, Jinju, Korea) or a Bruker Avance-III 800-MHz NMR spectrometer (KBSI, Ochang) equipped with a triple resonance cryogenic probe. All ^{1}H-^{15}N HSQC and imino proton spectra were obtained for complexes prepared by addition of DNA to 1 mM ^{15}N-labeled caZα_{PKZ} or addition of ^{15}N-labeled caZα_{PKZ} to 0.1–0.2 mM DNA One-dimensional (1D) NMR data were processed with either the program VNMRJ (Agilent, Santa Clara, CA) or FELIX2004 (FELIX NMR, San Diego, CA), whereas 2D data were processed with the program NMRPipe [13] and analyzed with the

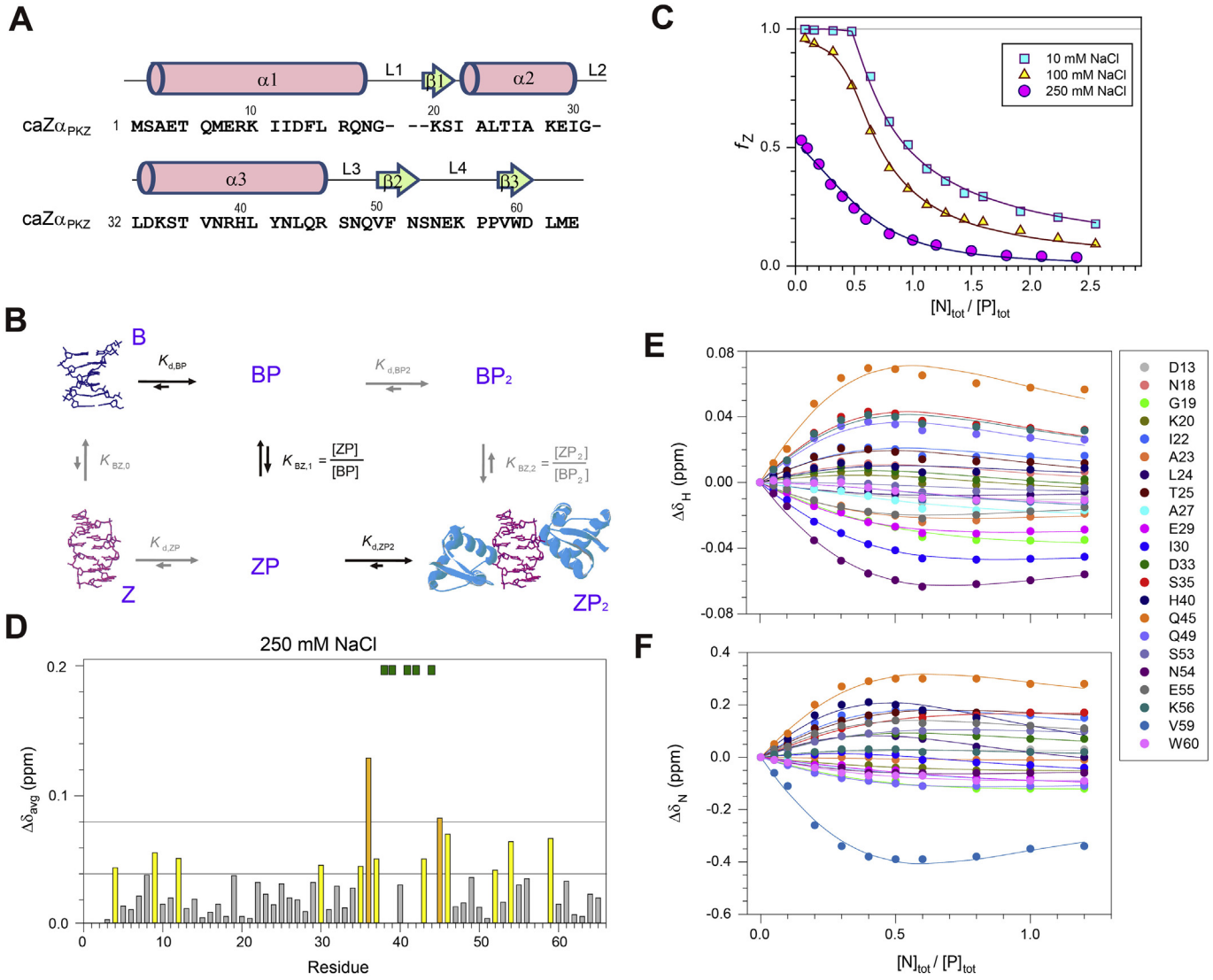

Fig. 1. (A) Sequence and secondary structure of caZα_{PKZ}. (B) Mechanism for the B–Z transition of a 6-bp DNA duplex by two molecules of Z-DNA binding protein. (C) Relative Z-DNA populations (f_Z) of dT(CG)$_3$ induced by caZα_{PKZ} at 10 (blue square) [12], 100 (yellow triangle) [12], or 250 mM NaCl (magenta circle, this study) as a function of $[N]_{tot}/[P]_{tot}$ ratio. (D) Histogram of the $\Delta\delta_{avg}$ of ^{15}N-labeled caZα_{PKZ} upon dT(CG)$_3$ binding at 250 mM NaCl [12]. (E) ^{1}H and (F) ^{15}N chemical shift changes of HSQC cross-peaks of caZα_{PKZ} upon dT(CG)$_3$ titration at 250 mM NaCl. Solid lines are the best fits to the equation described previously [12]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)