Journal of the Korean Magnetic Resonance Society **2015**, *19*, 95-98
DOI 10.6564/JKMRS.2015.19.2.095

Expression and Purification of the Helicase-like Subdomains, H1 and H23, of Reverse Gyrase from *A. fulgidus* for Heteronuclear NMR study

Mun-Young Kwon†, Yeo-Jin Seo†, Yeon-Mi Lee, Ae-Ree Lee, and Joon-Hwa Lee*

Department of Chemistry and RINS, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea

Received Aug 8, 2015; Revised Aug 22, 2015; Accepted Aug 25, 2015

Abstract Reverse gyrase is a hyperthermophile specific protein which introduces positive supercoils into DNA molecules. Reverse gyrase consists of an N-terminal helicase-like domain and a C-terminal topoisomerase domain. The helicase-like domain shares the three-dimensional structure with two tandem RecA-folds (H1 and H2), in which the subdomain H2 is interrupted by the latch domain (H3). To understand the physical property of the hyperthermophile-specific protein, two subdomains af_H1 and af_H23 have been cloned into *E. coli* expression vector, pET28a. The ^{15}N-labeled af_H1 and af_H23 proteins were expressed and purified for heteronuclear NMR study. The af_H1 protein exhibits the well-dispersion of amide signals in its ^{1}H/^{15}N-HSQC spectra and thus further NMR study continues to be progressed.

Keywords NMR, reverse gyrase, subdomain, protein purification, helicase-like, hyperthermophile

Introduction

Reverse gyrase is a catalytic enzyme which introduces positive supercoils into DNA molecules.[1-4] Reverse gyrase is the only enzyme that can be found in all hyperthermophilic organisms and it is thought to be responsible for the stabilization of DNA under extreme heat condition.[1-2]

Reverse gyrase consists of an N-terminal helicase-like domain and a C-terminal topoisomerase I domain.[2-3] The helicase-like domain includes an ATP binding site and shares the three-dimensional structure superfamily 2 (SF2) helicases, two tandem RecA-folds (H1 and H2) connected by linker (Fig. 1A).[3,5] The second RecA-fold H2 is interrupted by the so-call latch domain (H3) that shows homology to the transcription termination factor rho (Fig. 1A).[5] The crystal structure of the reverse gyrase from *A. fulgidus* suggested the mechanism for positive supercoiling that is based on the conformational change of the helicase-like domain as the initiation step.[6] This conformational change leads to a closure of the two RecA subdomains (H1 and H2) and then allows for release of the latch domain (H3).[6] It has been reported that such a conformational change occurs in SF2 helicases through the cooperative binding of ATP and nucleic acid substrates.[7]

Reverse gyrase recognizes destabilized regions of DNA double helix, which depend on temperature and DNA sequence. Interestingly, the reverse gyrase has very different temperature dependencies of stability and activity compared to its structural homologs. The optimal working temperature of reverse gyrase is 80 – 100 ^{o}C and it is able to efficiently produce highly positive supercoiled DNA at this temperature.[8] Although the structural studies explain well the

†These authors contributed equally to this work.

* Address correspondence to: **Joon-Hwa Lee,** Department of Chemistry and RINS, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea, Tel: 82-55-772-1490; Fax: 82-55-772-1489; E-mail: joonhwa@gnu.ac.kr

mechanism for the biological function of reverse gyrase, the molecular basis of the extremely high working temperature of this hyperthermophilic protein is not well understood.

Heteronuclear NMR spectroscopy is a suitable experimental tool to investigate the thermostability and structural flexibility of hyperthermophilic proteins as a function of temperature. In this study, we prepared the ^{15}N-labeled samples of the two subdomains H1 and H23 of reverse gyrase from *A. fulgidus* and confirmed the availabilities of these two proteins for heteronuclear NMR study by the ^{1}H/^{15}N-HSQC spectra.

Experimental Results

The regions encoding the H1 (D40 – Q238, af_H1) and H23 subdomains (V254 – Q499, af_H23) of reverse gyrase from *A. fulgidus* (*see* Fig. 1B) were purchased from Bioneer Co. (Seoul, Korea). The DNA encoding the af_H1 and af_H23 subdomains were cloned into pET28a using NheI/XhoI and NdeI/XhoI restriction sites, respectively (Fig. 1C and 1D) and DNA constructs were checked by DNA sequencing.

The af_H1 and af_H23 expression plasmids were transformed into *Escherichia coli* BL21(DE3) strain. Expression and purification steps are summarized in Fig. 2. A colony of transformed cells was picked and grown at 37 ^{o}C in minimal medium including ^{15}N-NH_4Cl as nitrogen source with 30 μg/ml kanamycin until A_{600} reached 0.6 – 0.7. Induction with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 4 hours at 37 ^{o}C led to the production of the expected proteins migrating to the expected molecular mass of the recombinant af_H1 and af_H23 helicase-like subdomains of *A. fulgidus* reverse gyrase on SDS-PAGE (lane 2 in Fig. 2A).

Cells were resuspended in 10 volumes of buffer L (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 10 mM imidazole, 1 mM PMSF), lysed by sonication, and centrifuged at 11,000 rpm for 60 min at 4 ^{o}C. The supernatant was applied to a Ni-NTA column (GE Healthcare co, Sweden) equilibrated with buffer L. The column was washed with a 20 mL of buffer W (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 10 mM imidazole). Elution was performed by applying a buffer E (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 1 M imidazole). The protein samples were eluted in first 3 mL of eluent fractions which was checked by 18 % SDS-PAGE (Fig. 2B). The sample fractions were concentrated in Amicon concentrator (Centricon 3K) and then loaded onto a gel filtration column (Sephacryl S-100, GE Healthcare, Sweden) on a GE AKTA FPLC to eliminate traces of remaining contaminants. Protein concentration was determined photochemically using the extinction coefficient at

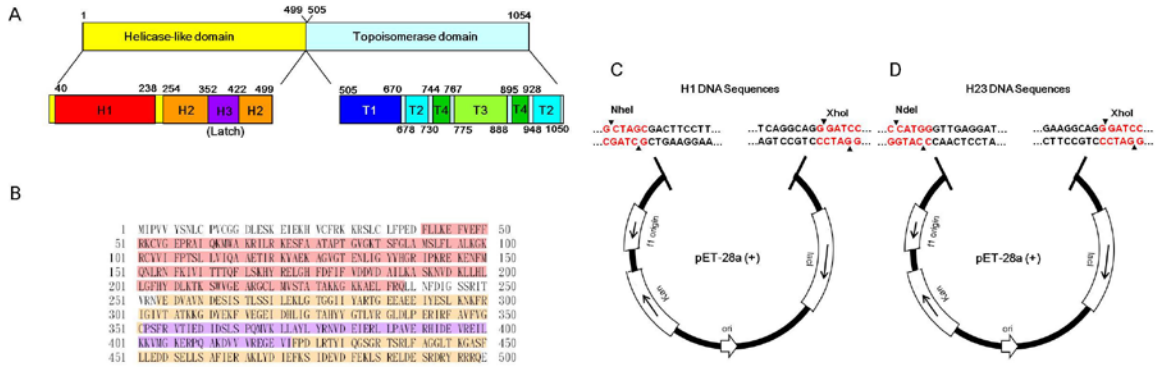

Figure 1. (A) Domain structure of reverse gyrase from *A. fulgidus*. (B) Amino acid sequence of the helicase-like domain of *A. fulgidus* reverse gyrase. Red, orange, and purple bars indicate the H1, H2, and H3 subdomains, respectively. (C, D) Plasmid constructs for expression of (C) the af_H1 and (D) af_H23 helicase-like subdomains.

280 nm of 20,065 $M^{-1}cm^{-1}$ for af_H1 and 14,900 $M^{-1}cm^{-1}$ for af_H23, respectively. We obtained about 4 and 2 mg of af_H1 and af_H23 subdomains subdomains of *A. fulgidus* reverse gyrase from 1 liter of culture, respectively.

For NMR experiments, the purified ^{15}N-labeled af_H1 and af_H23 subdomains of *A. fulgidus* reverse gyrase were concentrated to 0.5 mM in a 90 % H_2O/10 % D_2O buffer containing 10 mM sodium phosphate (pH 8.0) and 100 mM NaCl.

All NMR experiments were carried out on a Agilent DD2 700 MHz spectrophotometer (GNU, Jinju) equipped with *x*,*y*,*z*-axis pulsed-field gradient cold probe. NMR data were processed with the program NMRPIPE[9] and analyzed with the program Sparky.[10] 2D ^{1}H/^{15}N-HSQC spectra were acquired with 2,048 (H_N) × 64 (N) data points and 16 scans at 25 ^{o}C.[11,12] Spectral widths of 7,716 Hz (11 ppm) and 2,414 Hz (34 ppm) were used in the H_N and N dimensions, respectively.

Fig. 3 shows the ^{1}H/^{15}N-HSQC spectra of the af_H1 and af_H23 subdomains acquired at 25 ^{o}C. The amide signals of the af_H1 subdomain are well-resolved under our NMR buffer condition (Fig. 3A), indicating its well-folded monomeric structure. The ^{1}H/^{15}N-HSQC spectra of the af_H1 subdomain was not changed by heating up to 60 ^{o}C (*data not shown*). This means the af_H1 subdomain of *A. fulgidus* reverse gyrase is thermodynamically stable protein. Now further NMR study with ^{13}C,^{15}N-labeled af_H1 protein continues to be progressed.

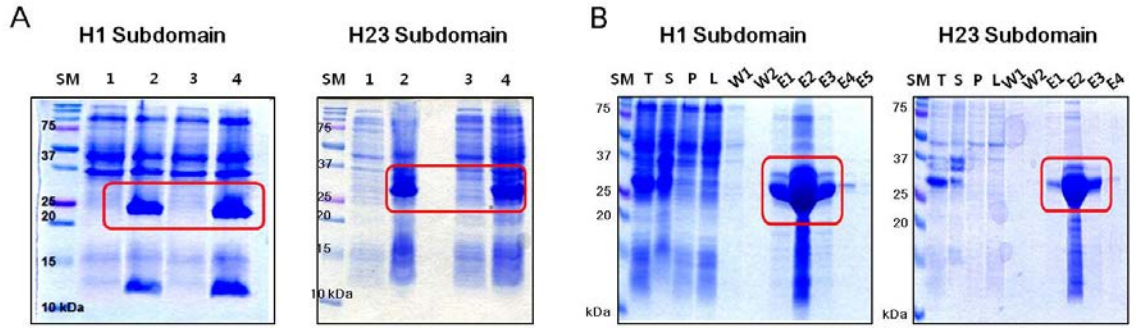

Figure 2. (A) Induction of recombinant af_H1 and af_H23 subdomains. Aliquots of cells before (*lanes 1 and 3*) and after IPTG induction (*lanes 2 and 4*) were analysed by 18 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE). (B) Purification of recombinant af_H1 and af_H23 subdomains by Ni-NTA affinity column. Aliquots of the purification steps of af_H1 (left) and af_H23 (right) were analysed by 18 % SDS-PAGE. *Lane T*, induced cells; *lane S*, supernatant after cell lysis; *lane P*, pallet after cell lysis; *lane L*, loading samples into Ni-NTA column; *lanes W1 and W2*, washing column with buffer W (each 10 mL); *lanes E1 – E5*, elution with buffer E (each 1 mL).

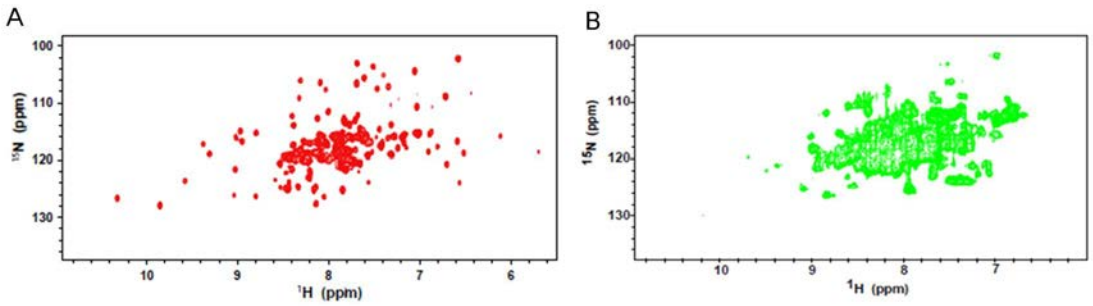

Figure 3. ^{1}H/^{15}N-HSQC spectra of (A) the H1 and (B) H23 subdomains of *A. fulgidus* reverse gyrase acquired at 25 ^{o}C.

The $^{1}H/^{15}N$-HSQC spectrum of the af_H23 shows the significant crowding of amide resonances in the central part of spectrum (Fig. 3B), indicating its oligomeric conformation. In order to perform heteronuclear NMR study on the af_H23 subdomain, we must find the optimal buffer condition in which the af_H23 protein exhibits the monomeric (or dimeric) conformation like af_H1 subdomain.

In summary, we prepared the plasmids expressing the af_H1 and af_H23 helicase-like subdomains of *A. fulgidus* reverse gyrase. The expressed ^{15}N-labeled af_H1 and af_H23 proteins were purified by Ni-NTA column and gel filtration chromatography. The af_H1 protein exhibits the well-dispersion of amide signals in its $^{1}H/^{15}N$-HSQC spectra. However, the recombinant af_H23 subdomain exhibited oligomeric conformation under our experimental condition. Now further NMR study with $^{13}C,^{15}N$-labeled af_H1 protein continues to be progressed.

Acknowledgements

This work was supported by the National Research Foundation of Korea Grant (2013-R1A2A2A05003837) funded by the Korean Government (MSIP). This work was also supported by a grant from Next-Generation BioGreen 21 Program (SSAC, no. PJ01117701), Rural Development Administration, Korea. We thank the GNU Central Instrument Facility for performing the NMR experiments.

References

1. C. De La Tour, C. Portemer, R. Huber, P. Forterre, and M. Duguet, *J. Bacteriol.* **173**, 3921 (1991)
2. M. Heine and S. B. C. Chandra, *J.Microbiol.* **47**, 229 (2009)
3. G. Perugino, A. Valenti, A. D'Amaro, M. Rossi, and M. Ciaramella, *Biochem. Soc. Trans.* **37**, 69 (2009)
4. M. Nadal, *Biochimie* **89**, 447 (2007)
5. Y. del Toro Duany, S. P. Jungblut, A. S. Schmidt, and D. Klostermeier, *Nucleic Acids Res.* **36**, 5882 (2008)
6. A. C. Rodriguez and D. Stock, *EMBO J.* **21**, 418 (2002)
7. B. Theissen, A. R. Karow, J. Kohler, A. Gubaev, and D. Klostermeier, *Proc. Natl. Acad. Sci. USA* **105**, 548 (2008)
8. T. S. Hsieh and J. L. Plank *J. Biol. Chem.* **281**, 5640 (2006)
9. F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, and A. Bax, *J. Biomol. NMR* **6**, 277 (1995)
10. T. D. Goddard and D. G. Kneller, SPARKY 3. University of California, San Francisco, CA. (2003)
11. J.-Y. Suh, T.- K. Yu, Y.- J. Yun, and K. O. Lee, *J. Korean Magn. Reson. Soc.* **18**, 1 (2014)
12. S. J. Park, *J. Korean Magn. Reson. Soc.* **18**, 47 (2014)