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Expression, Purification and Characterization of the BLM binding region of human Fanconi Anemia Group J Protein

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Abstract FANCJ is a DNA helicase which contributes genome stability by resolving G-quadruplex DNA from 5' to 3' direction. In addition to main ATPase helicase core, FANCJ has the protein binding region at its C-terminal part. BRCA1 and BLM are the binding partner of FANCJ and these protein-protein interactions contribute genomic stability and the proper response to replication stress. As the first attempt for studying FANCJ-BLM interaction, we prepared BLM binding region of FANCJ and characterized with CD and NMR spectroscopy. FANCJ (881-941) with N-ter 6xHis was purified as the oligomer. Secondary structure prediction based on CD data revealed that FANCJ (881-941) composed with β sheet, turn and coils. ^{1}H-^{15}N HSQC spectra showed nonhomogeneous peak intensities with less number of peaks comparing than the number of amino acids in the construct. It indicated that optimization should be necessary for detailed further structural studies.

Keywords Helicase, Protein-Protein interaction, CD, NMR

Introduction

Fanconi Anemia Group J (FANCJ) is one of the 15 complementation proteins in Fanconi Anemia which is an autosomal recessive disease with chromosomal instability.[1] It is a DNA helicase which resolves G-quadruplex (G4) structure from 5' to 3' direction with ATP hydrolysis. G4s are alternative DNA structures which appear frequently in guanine rich sequence such as telomere and gene promoters.[2-3] In this aspect, it has been known that FANCJ protects chromosomal structure and helps relaxing replication and transcription stress by resolving G4 structure.[4]

FANCJ consists of main ATPase helicase core domain and the protein interaction region at C-terminal part. FANCJ (888-1063) has been pointed as the Breast Cancer 1 (BRCA1) binding region.[5] Interestingly, it is overlapped with Bloom syndrome protein (BLM) binding region which is FANCJ (881-1060).[6] BRCA1 and FANCJ are both the tumor suppressor that involve on double strand break repair.[7] BLM is the one of the RecQ helicase which resolves G4 from 3' to 5' direction.[8-9] It is interesting that both FANCJ and BLM are able to unfold G4s but with the opposite direction while they interact each other. However, the details of interactions between FANCJ and BLM have not been studied yet. Previous study showed that FANCJ–BLM interaction is improved when cells are exposed to DNA damage.[7] FANCJ and BLM work together for helping cells handle with replication stress.[6] FANCJ-deficient cells, like BLM deficient cells, are sensitive to the replication inhibitor. The catalytic

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activity of FANCJ and its effect on the stability of BLM contribute to the preservation of genomic stability and a normal response to replication stress. [6]
In order to perform systematic study for FANCJ-BLM interaction, we firstly aimed to prepare and characterize BLM binding region of FANCJ. In this study, we cloned FANCJ (881-941) as the BLM Binding region. Overexpressed and purified protein was characterized with Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopy. [10-11] We found that FANCJ (881-941) was purified as the oligomer in gel filtration chromatography and β sheet rich protein as the result of CD analysis. ^{1}H-^{15}N HSQC spectra indicated that the further optimization should be necessary for detailed structural studies.

Experimental Methods

Construct cloning– Full size gene of human FANCJ was obtained from Addgene Inc. The DNA encoding FANCJ (881-941) was amplified by polymerase chain reaction (PCR) from FANCJ full gene. The PCR product was cloned into pET15b vector which has N-terminal 6xHis by using NdeI/XhoI restriction sites. DNA construct was confirmed with the DNA sequencing. It was transformed into *Escherichia coli* strain BL21 (DE3) strain.

Protein expression and purification– Cells were grown in LB medium at 37 °C and 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to the culture medium for the induction when the 600 nm optical degree is reached 0.5. Cells were cultured 5 hours more at 30 °C after induction. Cells were harvested by 15 minutes of centrifuge at 9,000 rpm at 4°C.
Cells were resuspended with the buffer containing 50 mM NaH_2PO_4 and 300 mM NaCl at pH 8.0. After the sonication, the protein was purified with Ni-NTA column. The mixture was loaded into the column and the flow through was collected. The column was washed with the wash buffer which is 50 mM NaH_2PO_4, 300 mM NaCl and 30 mM imidazole at pH 8.0. After the wash step, elution was performed with the elution buffer, 50 mM NaH_2PO_4, 300 mM NaCl and 300 mM imidazole at pH 8.0. Each fraction was analyzed with 15% SDS-PAGE Gel (Fig. 2A). Further purification was performed by gel extraction chromatography using AKTA pure and Hi-Load 16/600 75pg (GE Healthcare) with 20 mM Tris, 100 mM NaCl, 1mM DTT at pH 6.5.
^{15}N labeled proteins were purified from *E.coli* grown in the M9 media which containing ^{15}N labeled NH_4Cl. The cells were incubated at 30 °C for 5 hours after induction. Following purification steps are same with the purification of LB cultured protein.

Circular dichroism (CD)– CD experiments were performed in order to investigate secondary structure of FANCJ (881-941) on JASCO J-815 CD spectroscopy. All spectra were collected from 195 nm to 260 nm at 20 nm per minute and averaged after 3 times with spectral band width of 2 nm at 20 °C. The protein concentration was 45 uM and in 20 mM Tris and 100 mM NaCl buffer. The pH was either 6.5 or 7.5. The pH 5.5 buffer was made with 20 mM Na acetate and 100 mM NaCl.

NMR Spectroscopy– The NMR sample contained 0.3 mM ^{15}N-labeled FANCJ (881-941) in 20 mM Tris-HCl, 100 mM NaCl, 1 mM DTT at pH 6.5. ^{1}H - ^{15}N HSQC experiments was performed using 600 MHz Varian NMR spectrometer at 298 and 308 K (KBSI, Gwangju).

Results

Expression and purification of FANCJ (881-941) –It has been reported that the FANCJ and BLM physically and functionally interact *in vivo*.[6] With immunoprecipitation experiments, FANCJ (881-1060) was suggested as the BLM interaction site (Fig.1). We firstly tried to clone and express FANCJ (881-1060). However, the expression level was exceptionally low and it hindered further analysis of that region (*data not shown*). Interestingly, FANCJ (1-990) also could bind BLM while FANCJ (1-660) could not.[6] Therefore, we cloned FANCJ

(881-941) which contain the overlapped region of FANCJ (881-1060) and FANCJ (1-900), from 881 to 900.

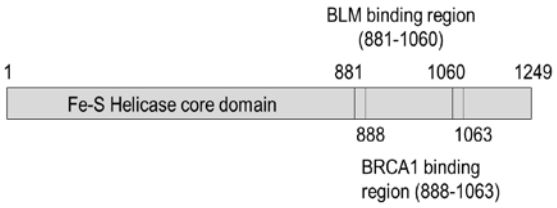

Figure 1. Domain structure of human FANCJ protein. BLM binding region is located in the c-terminal part and overlapped with BRCA1 binding region.

With N-terminal 6xHis tag, FANCJ (881-941) was overexpressed and purified. Fig.2 shows purification steps with SDS-PAGE image of Ni-NTA affinity column and the elution profile of the gel filtration chromatography.

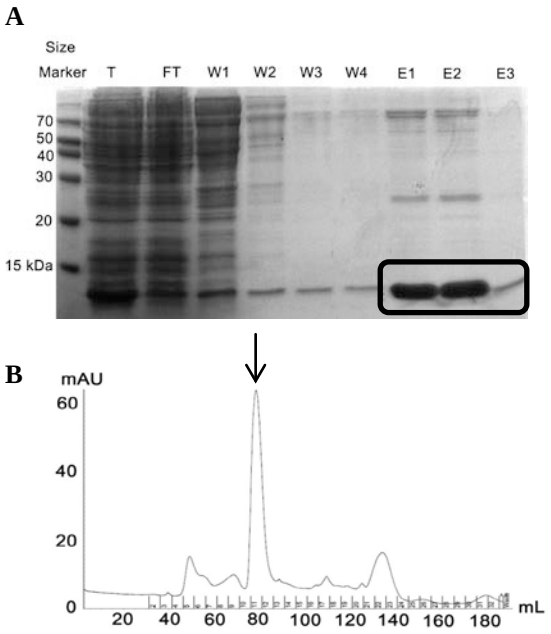

Figure 2. (A) Purification of FANCJ (881-941) with Ni-NTA affinity column. *Lane* T, induced cells, *lane* FT, flow through after loading sample, *lane* W1-W4, washing column with wash buffer (each 15 mL), *lane* E1-E3, elution with elution buffer (each 5 mL). (B) Elution profile of FANCJ (881-941) with Hi-Load 16/600 75pg gel filtration column. The arrow indicates the elution of FANCJ (881-941).

Even though FANCJ (881-941) was partially insoluble, we were able to purify the enough amount of the soluble protein for the further CD and NMR Spectroscopy analysis. However, the protein was purified as the oligomer which was eluted earlier than predicted (Fig 2(B)).

CD spectroscopy– In order to characterize the secondary structure of FANCJ (881-941), CD spectoscopy was applied. Figure 3(A) shows CD spectra of FANCJ (881-941) in three different pH conditions (pH 5.5, pH 6.5, and pH 7.5). Three profiles were almost similar one another with the minimum ellipticity at around 200 nm. The secondary structure prediction was performed with BeStSel method.[12] Estimated secondary structures were composed with 43.2 % of Antiparallel β strand, 16.1 % of turn and 40.7 % of others. The RMSD between the measured value and predicted one was 0.1443. Figure 3(B) shows the comparison of the meausred value at pH 6.5 and predicted value.

NMR spectroscopy– We performed ^{1}H-^{15}N HSQC experiments of FANCJ (881-941) at pH 6.5 with two different temperature (298 and 308 K) (Figure 4). Two spectra were quite similar each other with narrow dispersion in ^{1}H dimension. Also, peak intensities are not homogeneous. There are around 20 strong peaks and the similar numbers of weak peaks. Comparing with number of amino acids in FANCJ (881-941), there were less number of peaks in the spectra.

Discussion

In this study, FANCJ (881-941) as the binding region of BLM was expressed, purified and characterized wih CD and NMR spectroscopy. Interestingly, the protein was purified as the soluble oligomer in the physiological condition. The analysis of CD spectrum implies that the protein is mainly composed with antiparallel β strand and random coil. It has been known that the fragments or designs of β structure usually tends to aggregate.[13] In this aspect, FANCJ

(881-941) can be considered as the β-sheet rich fragment which forms oligomeric state. ^{1}H-^{15}N HSQC spectra which shows nonhomogeneous peak intensities and less numbers of peaks than predicted support the idea that the protein are not well structured. Further optimization of the construct for solving difficulties of structural studies are on going.

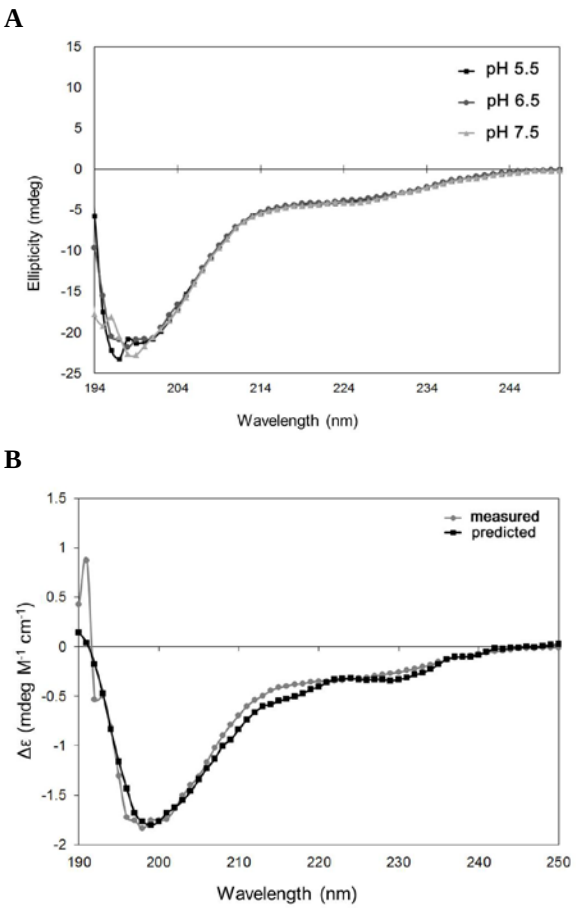

Figure 3. (A) CD spectra of FANCJ (881-941) recorded in three different pHs (pH 5.5, pH 6.5, and pH 7.5). (B) Comparison of CD spectra of FANCJ (881-941) at pH 6.5 and predicted value with BeStSel method.

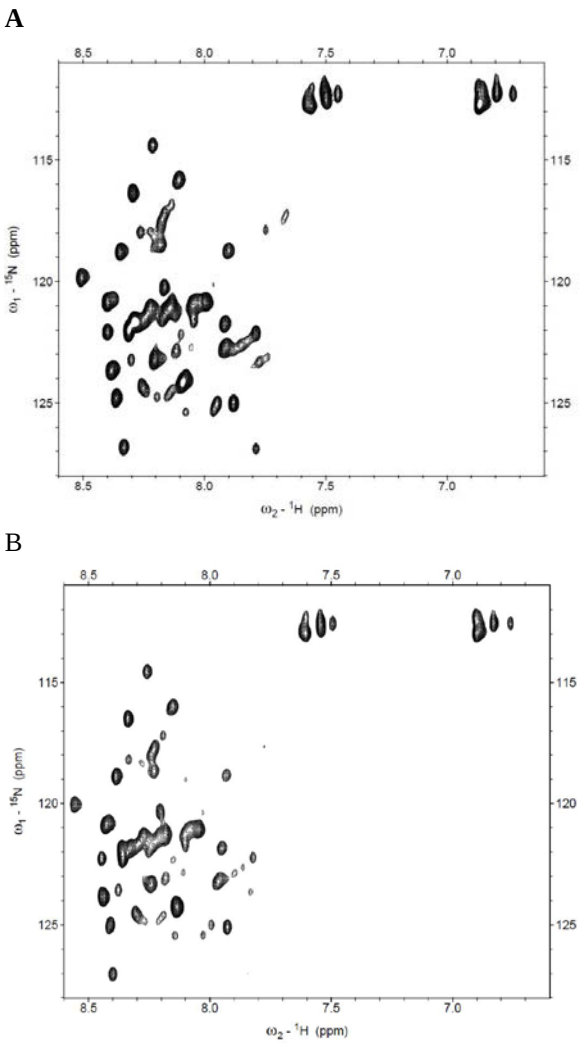

Figure 4. ^{1}H-^{15}N HSQC spectra of FANCJ (881-941) at (A) 308 K (B) 298 K.

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