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Expression and Preparation of Periostin FAS1 Domains for NMR Structure Determination

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Abstract Periostin, a component of extracellular matrix (ECM) protein, is produced and secreted by the fibroblasts that are involved in chronic allergic inflammation diseases and various types of human cancers. Periostin protein is composed of multiple domains including four FAS1 domains which play important roles in cell adhesion and tumor metastasis by interacting with integrins. In spite of their important biological role, the structural information of periosin FAS1 domains was not revealed yet. Recently we systemically prepared various constructs of the FAS1 domains and tried to express them in *E. coli*. Of them, only single FAS1-II and -IV domains were highly soluble. Circular dichroism (CD) and nuclear magnetic resonance (NMR) studies revealed that the FAS1-IV domain might be suitable for three-dimensional structure determination using NMR spectroscopy.

Keywords Periostin, FAS1, Integrin, Cancer metastasis, Chronic inflammation

Introduction

Periostin, a 90 kDa cell adhesion protein secreted in extracellular matrix (ECM) by fibroblasts, was originally identified in mouse osteoblastic cell line.[1-2] Periostin is mostly expressed in the periosteum and periodontal ligament, suggesting that it is involved in the bone formation and heart development.[3-4] Periostin expression was also observed in normal organs and tissues as well as a variety of tumors, including thyroid carcinoma, breast cancer, colon cancer, ovarian cancer, and pancreatic cancer.[5-7] In addition, periostin is also involved in atopic dermatitis and bronchial asthma as chronic inflammatory diseases caused by fibroblast stimulus.[8-9] Periostin is composed of an N-terminal signal sequence, followed by a cysteine-rich EMILIN (EMI) domain, four homologous fasciclin (FAS1) domain, and a C-terminal hydrophilic variable domain. The signal sequence of the N-terminal domain promotes periostin secretion and controls the cell function by binding to plasma membrane receptor through its FAS1 domains.[10] The EMI domain of periostin consists of about 55 residues with 6 cysteines and is involved in the formation of multimers in non-reducing conditions.[11] The FAS1 domains of periostin are composed of approximately 150 amino acids and homologous to the transforming growth factor-β-induced protein as well as an insect cell adhesion protein fasciclin I.[12] In addition, FAS1 domains play an essential role in metastasis of cancer cell and chronic inflammatory by interacting with cell surface receptors such as integrin αvβ3, αvβ5, and α6β4. The C-terminal variable domain contains a proteolytic cleavage site and regulates the cell-matrix organization.[13]

Periostin binds to the several integrins which are heterodimeric transmembrane receptors activating Akt/PKB and FAK-mediated signaling pathways. The binding of periostin to integrins can promote cellular survival, invasion, metastasis, and

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angiogenesis via activation of signaling pathway in cancer cells.[14] In addition, periostin promotes chronic allergic inflammation such as bronchial asthma and atopic dermatitis by interacting with integrin on kerathinocytes.[15]

Periostin can be considered as a biomarker and therapeutic target for detection of cancer and allergic inflammation skin disease.[14,15] Although the great attention has been shown to the role of periostin, the structure of FAS1 domain was not reported yet. Therefore, the three-dimensional (3D) structure of FAS1 domain of periostin will help to understand the biological functions of periostin.[16,17] In this study, we report the preparation and preliminary structural studies of FAS1 domains of periostin using circular dichroism (CD) and nuclear magnetic resonance (NMR) for 3D structure determination of FAS1 domain of human periostin.

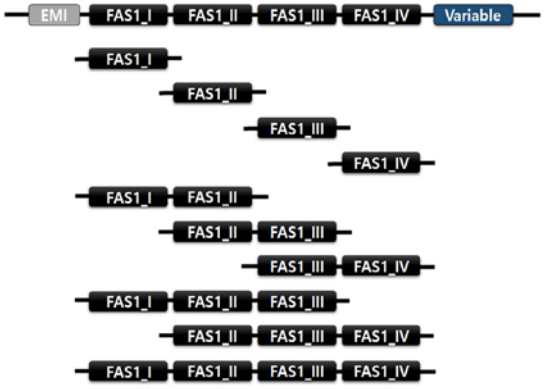

Figure 1. Scheme of FAS1 domain constructs of periostin for cloning. Four single, three double, two triple, and one quadruple fragments of FAS1 domain.

Experimental Methods

Cloning and expression of FAS1 domains of Periostin- Ten different constructs of FAS1 domains of human periostin (Fig. 1) were cloned into the expression vector, pHIS2 containing hexa-histidine tag and TEV protease cleavage site at N-terminus. The amplified PCR products containing NcoI and XhoI restriction enzyme site were ligated at room temperature for 1 h. The expression vector containing the recombinant constructs confirmed by DNA sequencing were transformed into *E. coli* BL21 (DE3) and cultivated in LB media at 37°C. When the OD_{600} was reached to 0.6, the cultivated cells were induced by adding 0.5 mM IPTG at 15°C for 20 h. The cell was harvested by centrifugation and sonicated in a lysis buffer (0.1 mM PMSF, 20 mM imidazole, 20 mM β–mercaptoethanol, 1X PBS pH 7.4). The cell lysate was separated by centrifugation with 15000 rpm at 4°C for 30 min. All expressed proteins were analyzed using SDS-PAGE for their expression and solubility.

Purification of FAS-II and –IV domains- The supernatant of lysate was loaded onto Ni-NTA open column. The bound protein was eluted with 50, 100, 150, 200, 250, and 300 mM imidazole in lysis buffer. The His-tagged protein was cleaved with TEV protease at 4°C for 16 h. To remove the His-tag and TEV protease, protein was purified with 2nd Ni-NTA open column and dialyzed using anion exchange buffer A (20mM Tris, 10 mM NaCl pH 8.0), The proteins were further purified by anion exchange chromatography (HiTrap Q, GE Healthcare) and gel filtration chromatography (HiPrep 16/60 sephacryl, GE Healthcare). The purified protein was concentrated by centrifugal filter (Merk) and dissolved in the final buffer (10 mM NaH_2PO_4, 50 mM NaCl, pH 7.0).

Stable isotope (^{15}N) labeling of FAS1-IV domain- For heteronuclear single quantum correlation (HSQC) NMR experiment, the expression construct of FAS1-IV was cultivated in M9 minimal medium with $^{15}NH_4Cl$ (Cambridge Isotope Lab) at 37°C. The grown cells were induced for 20 h with 0.5 mM IPTG at 15°C.

Circular dichroism (CD) spectroscopy- The CD spectra of FAS1 domains were measured by using a CD spectrometer (Jasco, J-815) in 10 mM NaH_2PO_4, 50 mM NaCl pH 7.0 at 20°C. The data were acquired from 260 to 190 nm by averaging three scans with molar ellipticity.

NMR spectroscopy- 1D and 2D NMR spectra of FAS1 domain were obtained on a Bruker and Varian 600 MHz NMR spectrometers with 10% D_2O for the frequency lock. The 2D ^{1}H-^{15}N HSQC of FAS1-IV domain was processed using NMRPipe and analyzed using NMRView software.

Results and Discussion

Ten different constructs designed from four FAS1 domains of periostin were prepared (Fig. 1) and expressed in *E. coli*. We confirmed that all FAS1 constructs were highly expressed. However only FAS1-II and -IV domains were expressed into soluble fractions (Fig. 2A and 3A).

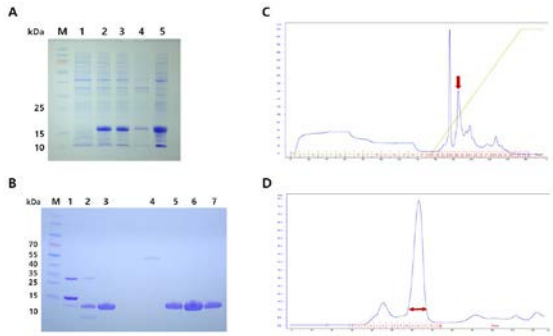

Figure 4. Purification of FAS1-II domain of periostin. (A) SDS-PAGE analysis of expression of FAS1-II domain. Lane 1, -IPTG; 2, +IPTG; 3, lysis; 4, lysis pellet; 5, lysis supernatant. (B) SDS-PAGE analysis of purification of the FAS1-II. Lane 1 and 2 are for before and after TEV cleavage. Lane 3 is for 28 fraction of anion-exchange chromatography. Lane 5 to 7 are for 11 to 13 fractions of gel filtration chromatography. (C) Chromatogram of anion-exchange and (D) gel filtration chromatography.

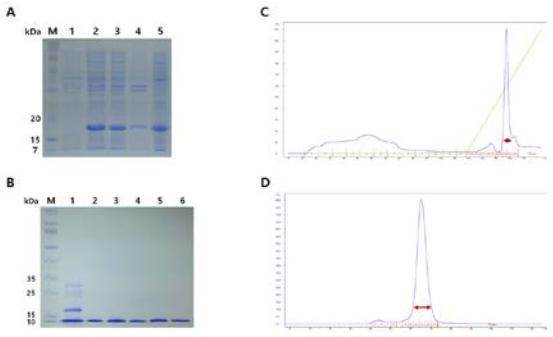

Figure 3. Purification of FAS1-IV domain of periostin. (A) SDS-PAGE analysis of expression of FAS1-IV domain. Lane 1, -IPTG; 2, +IPTG; 3, lysis; 4, lysis pellet; 5, lysis supernatant. (B) SDS-PAGE analysis of purification of FAS1-IV. Lane 1 is for unbound fraction of 2nd Ni-affinity chromatography. Lane 2 to 3 are for 22 to 23 fractions of anion-exchange chromatography. Lane 4 to 6 are for 10 to 12 fractions of gel filtration chromatography. (C) Chromatogram of anion-exchange and (D) gel filtration chromatography.

FAS1-II and -IV were purified by 1st Ni-affinity chromatography, followed by TEV cleavage and 2nd

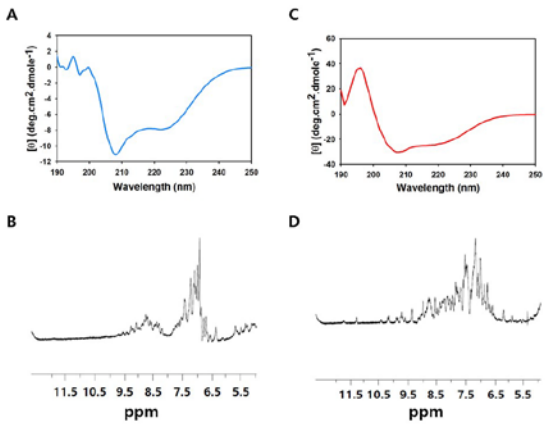

Figure 2. Structural characterization of FAS1-II and -IV domains. CD spectra of FAS1-II (A) and FA1-IV (C). 1D proton NMR spectra of FAS1-II (B) and FAS1-IV (D).

Ni-affinity chromatography. The molecular weight of FAS1-II and -IV were 15 and 15.7 kDa, respectively, which was confirmed by SDS-PAGE analysis (Fig. 2 and 3). To obtain pure FAS1-II and -IV proteins, we performed further purification steps using anion-exchange and gel filtration chromatography. Each purification results were analyzed by SDS-PAGE (Fig. 2B and 3B). The results indicate that the pure and stable FAS1-II and -IV proteins were obtained for the structural study.

To obtain a preliminary structural information of FAS1-II and -IV, CD and 1D ^{1}H NMR experiments were performed with the purified proteins. The CD spectra of FAS1-II (69 μM) and –IV (9.5 μM) showed that the purified proteins are comprised of a mixture of α–helix and β–sheet predicted by DichroWeb server based on CD spectra (Fig. 4A and 4C). Although the FAS1-IV has a lower concentration, the intensity of CD spectrum of FAS1-IV was much stronger than that of FAS1-II. In addition, the CD spectra suggested that FAS1-IV has higher secondary structure contents than FAS1-IV. These results suggest that the FAS1-IV domain is well folded than FAS1-II domain without soluble aggregation and denaturation.

Furthermore, the 1D ^{1}H NMR experiments of FAS1-II (0.5 mM) and -IV (0.1 mM) showed that the FAS1-IV displays a better dispersed spectrum than FAS1-II, suggesting that the FAS1-II domain is more disordered than the FAS1-IV domain (Fig. 4B and 4D). For the 3D structure determination of FAS1-IV,

we initially collected a 2D ^{1}H-^{15}N HSQC spectrum of FAS1-IV (Fig. 5). The total number of observed peaks in the HSQC spectrum was 156, which is slightly lower than the expected number of peaks (158). This may result from some peak overlapping or broadening. Although the intensity of some of peaks in the HSQC spectrum is not homogeneous, the overall quality of the spectrum seems to be good enough to solve the 3D structure. Therefore, we are currently trying to determine the 3D structure of FAS1-IV domain using NMR spectroscopy.

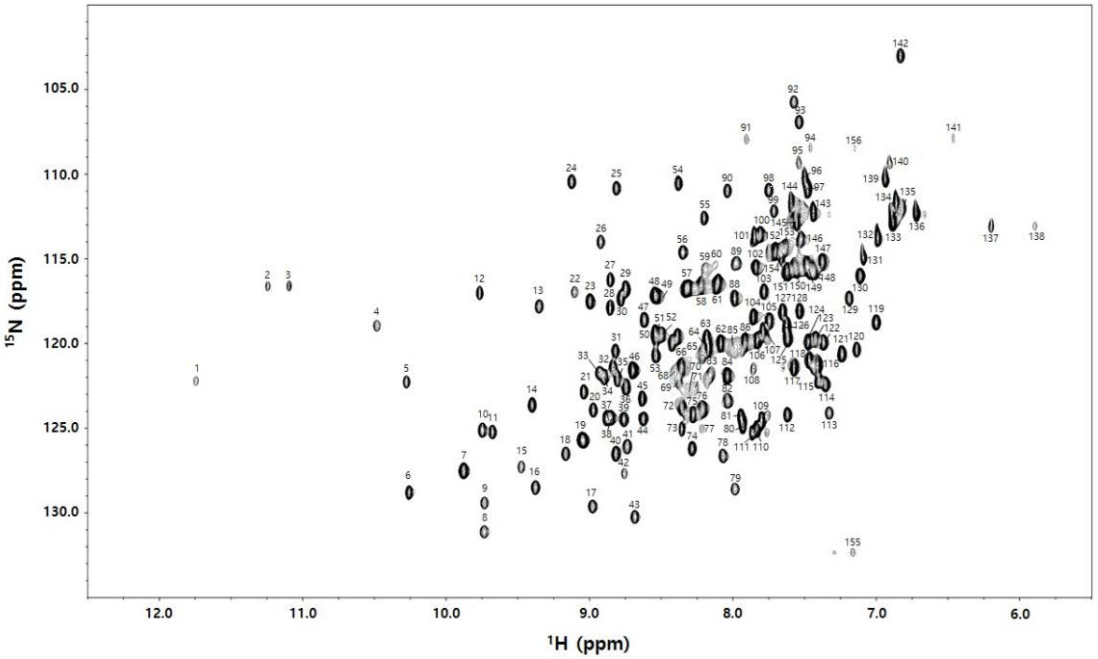

Figure 5. 2D ^{1}H-^{15}N HSQC spectrum of FAS1-IV domain of periostin collected on Varian 600 MHz.

Acknowledgements

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