# *$^{1}H$, $^{13}C$, and $^{15}N$ resonance assignments of FAS1-IV domain of human periostin, a component of extracellular matrix proteins*

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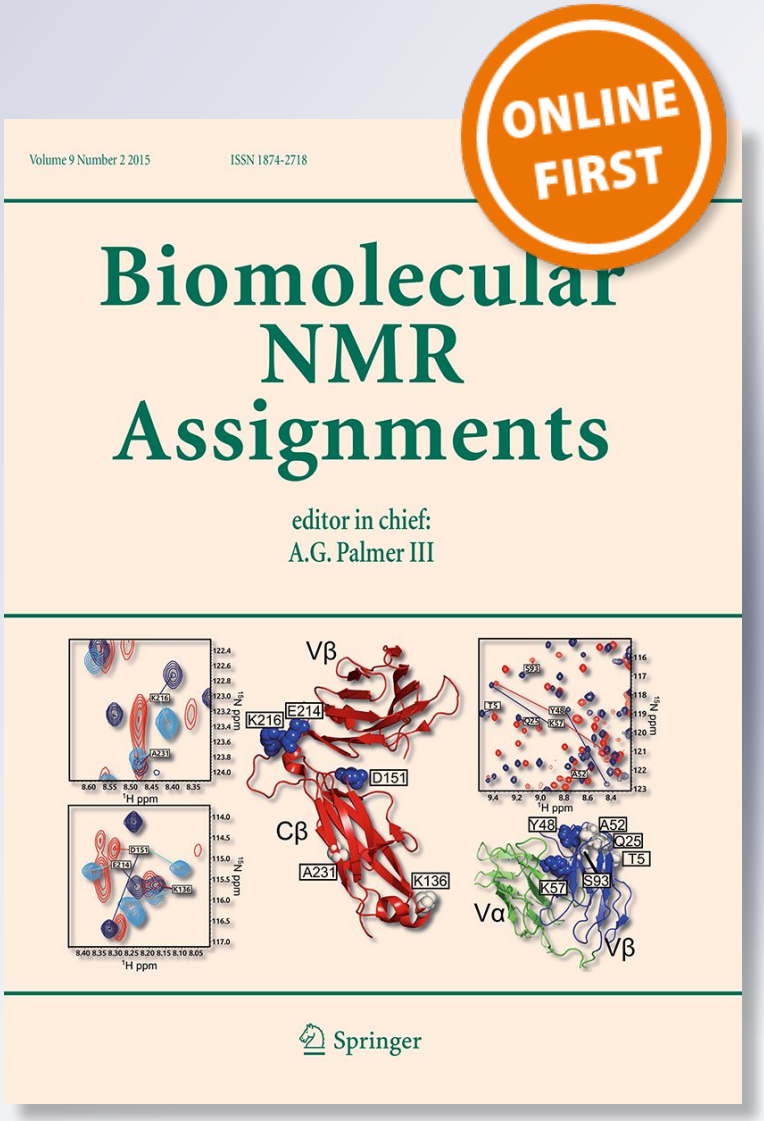


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ARTICLE

# $^{1}$H, $^{13}$C, and $^{15}$N resonance assignments of FAS1-IV domain of human periostin, a component of extracellular matrix proteins

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**Abstract** Periostin, an extracellular matrix protein, is secreted by fibroblasts and is overexpressed in various types of cancers. The four internal repeat fasciclin 1 (FAS1) domains of human periostin play crucial roles in promoting tumor metastasis and progression via interaction with cell surface integrins. Among four FAS1 domains of human periostin, the fourth FAS1 domain (FAS1-IV) was prepared for NMR study, since only FAS1-IV was highly soluble, and showed a well-dispersed 2D $^{1}$H-$^{15}$N HSQC spectrum. Here, we report nearly complete backbone and side chain resonance assignments and a secondary structural analysis of the FAS1-IV domain as first steps toward the structure determination of FAS1-IV of human periostin.

**Keywords** NMR resonance assignment · Periostin · Extracellular matrix · FAS1 domain

## Biological context

Periostin is an extracellular matrix protein that was originally identified in mesenchymal cells, such as osteoblasts, osteoblast-derived cells, periodontal ligament cells, and periosteal cells (Bruder et al. 1998; Horiuchi et al. 1999; Suzuki et al. 2004). Periostin has since been widely found in various tissues and organs, including the aorta, stomach, uterus, periodontal ligaments, cardiac valves, and breast (Tai et al. 2005). It seems to be involved in a broad range of cellular functions. Periostin has at least two major biological roles; one is fibrillogenesis, which occurs in the matrix, and another is cell migration (Kudo 2011). Recent accumulated studies show that periostin is involved in many basic biological processes, such as cell proliferation, cell invasion, and angiogenesis, and may also be involved in regulating a diverse set of cancer cell activities that contribute to tumorigenesis, cancer progression, and metastasis (Li et al. 2015). Furthermore, periostin has also been revealed to be involved in chronic inflammatory diseases, such as atopic dermatitis and asthma (Masuoka et al. 2012). Periostin can activate Akt/PKB and FAK-mediated signaling pathways by interacting with integrins on cancer cells. This activated signaling promotes cell survival, angiogenesis, invasion, metastasis, and epithelial-mesenchymal transition (Morra and Moch 2011). High expression of periostin has been detected in many solid tumors, and its expression correlates with tumor progression and metastases of cancer. Thus, in clinical practice, periostin can be considered as a biomarker and therapeutic target for the detection and treatment of cancers as well as chronic inflammatory diseases.

Periostin is a 90-kDa protein, which consists of an amino-terminal signal sequence, followed by an EMILIN-like (EMI) domain, four repeated fasciclin 1 (FAS1) domains, and a carboxyl-terminal variable domain (Bruder et al. 1998; Horiuchi et al. 1999; Takeshita et al. 1993). The EMI domain, rich in cysteine, is involved in protein–protein interaction or protein multimerization in non-reducing conditions (Doliana et al. 2000). The FAS1 domain is composed of about 150 amino acids (Litvin et al. 2005) and acts as a ligand for the integrin receptors αvβ3 and αvβ5, which initiate crosstalk with receptor tyrosine kinases such as EGFR and VEGF (Ghatak et al. 2014). The C-terminal variable domain undergoes alternative splicing, resulting in seven

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isoforms in humans that can be detected in various cancers, such as pancreatic, colon, and breast cancer (Hoersch and Andrade-Navarro 2010). Periostin can be posttranslationally modified by a vitamin K-dependent γ-glutamylcarboxylase (Coutu et al. 2008), since periostin contains 28 potential sites of carboxylation. However, the biological role of this gamma carboxylation is currently unknown.

Although periostin plays a wide variety of critical roles in many fundamental biological processes, there is not yet any published structural information on periostin domains. In this study, we successfully prepared the fourth FAS1 domain (FAS1-IV) of human periostin for NMR structural study. The resonance assignment of backbone and side chain of FAS1-IV was done using multi-dimensional NMR spectroscopy and the secondary structures of FAS1-IV were predicted by analyzing the NMR assignment data.

## Methods and experiments

### Cloning, expression, and protein purification of FAS1-IV domain

The expression and purification of the FAS1-IV domain of human periostin was performed as previously described (Yun et al. 2016). The gene encoding the FAS1-IV domain (residues 496–632) was amplified by PCR from human periostin cDNA using the following primers: forward 5′-AAA CGCCCATGGAGAAATCCCTCCATGAAAAG-3′ and reverse 5′-AAACGCCTCGAGTTAGTCTGCTGGATA GAGGAG-3′. The PCR product was cloned into a pHIS2 expression vector between NcoI and XhoI restriction sites. The FAS1-IV domain was overexpressed in BL21(DE3) with 0.5 mM IPTG for 20 h at 15 °C. The harvested cells were resuspended in lysis buffer, containing 20 mM Tris (pH 8.0), 0.5 M NaCl, 20 mM imidazole, and 0.1 mM PMSF, and lysed by sonication. The supernatant was loaded onto a Ni-NTA open column and the bound protein was eluted with 0.2 M imidazole in lysis buffer. The eluted protein was dialyzed against buffer A (20 mM Tris (pH 8.0) and 10 mM NaCl), and was treated with TEV protease to remove the N-terminal His-tag. To further purify, the cleaved FAS1-IV domain was reloaded onto the Ni–NTA open column and the unbound protein was purified using a HiTrapQ anion exchange column (GE Healthcare) with linear gradient from 0 to 0.5 M NaCl in buffer A containing 0.1 mM PMSF and 1 mM EDTA. The eluted protein was further purified using a HiPrep 16/60 gel filtration column (GE Healthcare) with buffer B (10 mM $NaH_2PO_4$ (pH 7.0), 50 mM NaCl, 0.1 mM PMSF, and 1 mM EDTA). The $^{15}N$- and $^{13}C/^{15}N$-labeled FAS1-IV proteins were expressed in M9 medium supplemented with $^{15}NH_4Cl$ and $^{13}C$-glucose.

### NMR spectroscopy

All NMR experiments were performed at 288 K using Bruker Avance 700, 800, and 900 MHz spectrometers at Korea Basic Science Institute (KBSI). Samples were prepared in gel filtration buffer containing 10 μM DSS, 0.04% $NaN_3$, and 10% $^2H_2O$/90% $H_2O$ or 100% $^2H_2O$. Backbone resonance assignments were obtained from 3D HNCA (Grzesiek and Bax 1992), HN(CO)CA, HNCACB (Wittekind and Mueller 1993), CBCA(CO)NH (Grzesiek and Bax 1992), and HNCO (Kay et al. 1990). Sidechain resonance assignments were based on 3D CCH-TOCSY, HCCH-COSY, and HCCH-TOCSY (Bax et al. 1990). $^1H–^{13}C$ HSQC (Palmer et al. 1991), $^{13}C$-NOESY-HSQC, $^{15}N$-TOCSY-HSQC, and $^{15}N$-NOESY-HSQC (Marion et al. 1989; Zuiderweg and Fesik 1989) were used for assignment of the side chain and aromatic region. All NMR spectra were processed and analyzed using the programs NMRPipe (Delaglio et al. 1995) and NMRViewJ (Johnson and Blevins 1994).

### Resonance assignments and data deposition

The FAS1-IV domain of human periostin comprises residues 496–632 with three additional residues (Gly-Ala-Met) at the N-terminus. The $^1H–^{15}N$ HSQC spectrum revealed that the FAS1-IV domain is well folded in NMR buffer based on the signal dispersion and homogenous intensity of resonances (Fig. 1). Almost all (98.3%) backbone resonances ($^1H$, $^{15}N$, $^{13}Cα$, $^{13}C′$) were assigned, except for two N-terminal residues, Gly574, Asn605, Glu606, and five prolines. A total of 130 resonances out of the 135 expected non-proline residues were assigned. Additionally, 93.2% of the observable sidechain resonances were assigned. The secondary structure of FAS1-IV domains was predicted using the TALOS-N program (Shen and Bax 2013) based on the backbone HN, N, CA, CB, and C′ chemical shifts in FAS1-IV. Figure 2 shows that the secondary structure of FAS1-IV is composed of six α-helices (residues 499–504, 510–518, 522–525, 538–540, 546–554, and 556–564) and six β-strands (residues 530–535, 581–585, 592–598, 601–604, 611–616, and 620–624). The primary sequence of FAS1-IV of periostin is homologous to those of FAS1 domains of *Drosophila melanogaster* (PDB ID 1O70) and transforming growth factor beta-induced protein (TGFβIp) (PDB ID 2LTB). As a result, the secondary structure of FAS1-IV of periostin is similar to those of FAS1 domains of other proteins. These data are in good agreement, which indicates that the conformation of FAS1-IV of periostin is similar to that of FAS1 domains found in other proteins. The $^1H$, $^{13}C$, and $^{15}N$ resonance assignments of FAS1-IV have been deposited to the BioMagResBank database under accession number 36039.

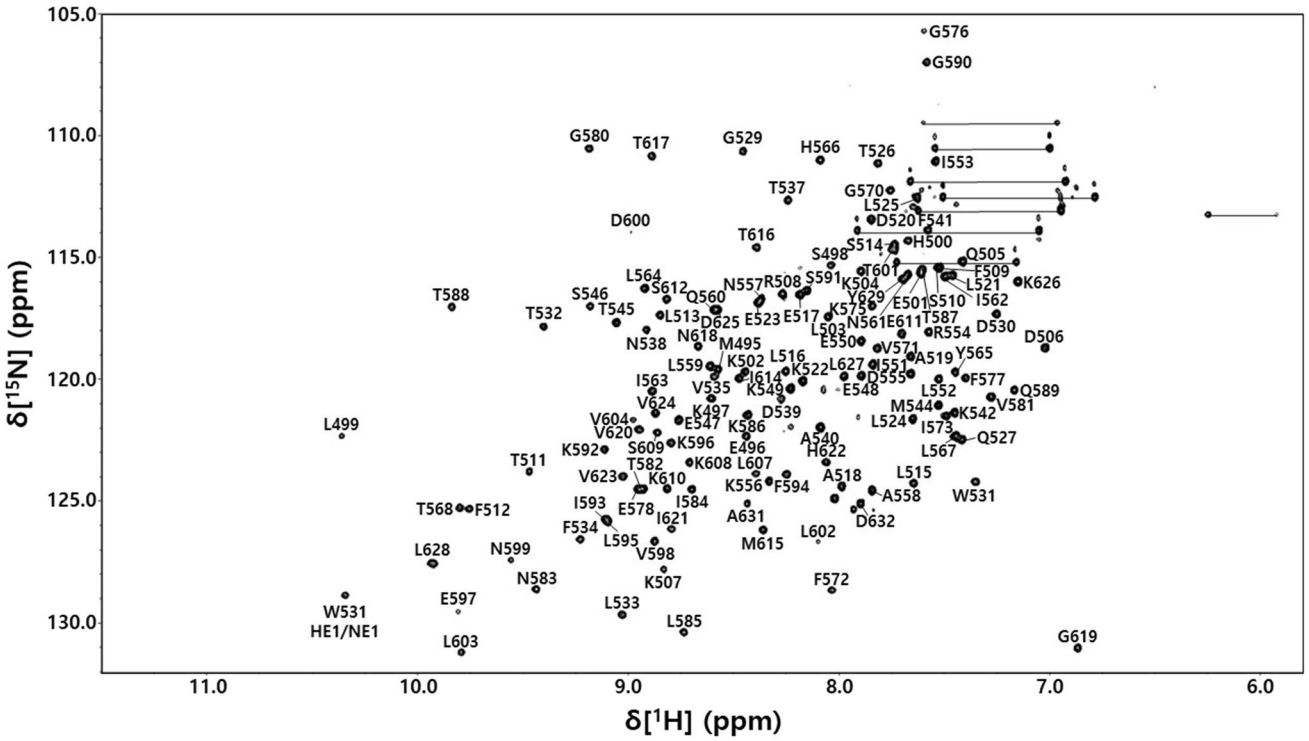


**Fig. 1** $^{1}$H–$^{15}$N HSQC spectrum of FAS1-IV of human periostin recorded at 288 K on a Bruker 900 MHz spectrometer. Residue numbers are labeled on the crosspeaks

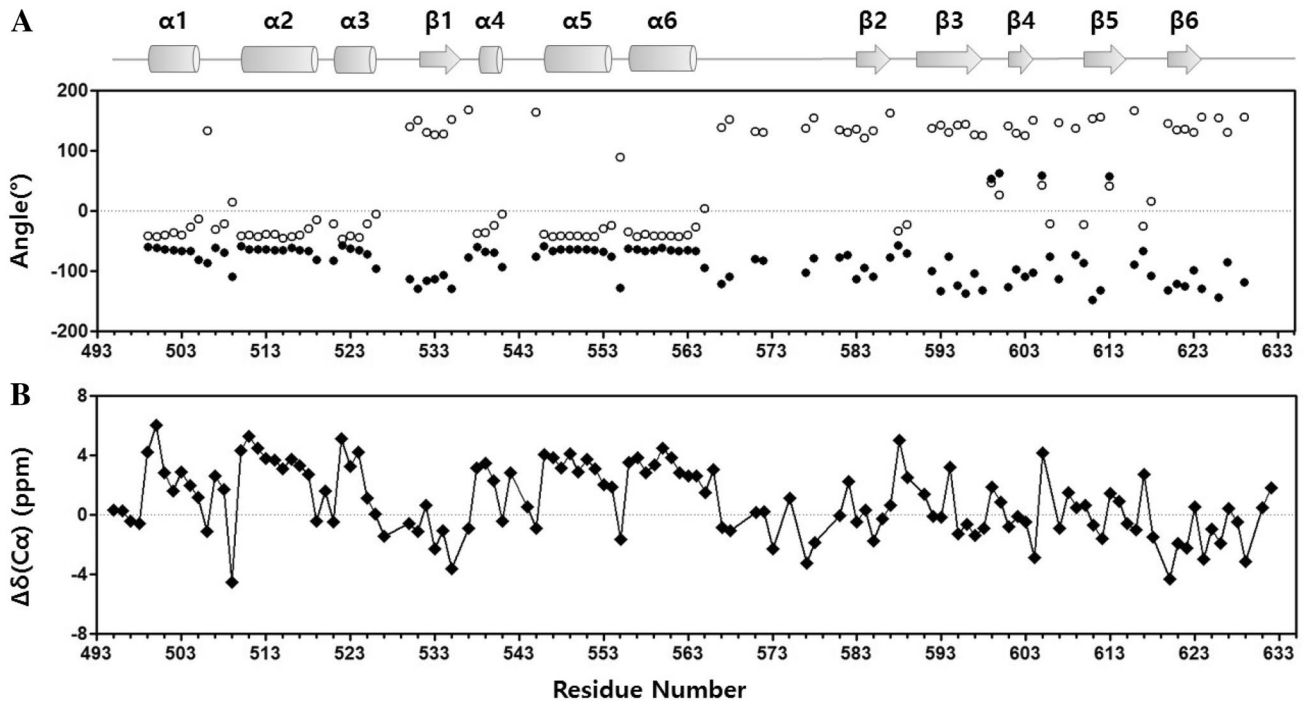


**Fig. 2** Backbone torsion angles of FAS1-IV of human periostin were predicted from the chemical shifts using TALOS-N(A). The filled and open circles indicate phi angles (φ) and psi angles (ψ), respectively. A schematic secondary structure is shown above panel (**A**), indicated with cylinders (α-helices) and arrows (β-strands). Chemical shift deviations of the backbone atom $^{13}$Cα value were calculated from chemical shift index values (**B**)

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