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Letter to the Editor: NMR assignment of the SH2 domain from the human feline sarcoma oncogene FES

Anna Scott[a,**], David Pantoja-Uceda[a], Seizo Koshiba[b], Makoto Inoue[b], Takanori Kigawa[b], Takaho Terada[b,c], Mikako Shirouzu[b,c], Akiko Tanaka[b], Sumio Sugano[d], Shigeyuki Yokoyama[b,c,e] & Peter Güntert[a,*]

[a]*Tatsuo Miyazawa Memorial Program, RIKEN Genomic Sciences Center, 1-7-22, Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan;* [b]*RIKEN Genomic Sciences Center, 1-7-22, Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan;* [c]*RIKEN Harima Institute at SPring-8, 1-1-1 Kouto, Mikazuki, Sayo, Hyogo 679-5148, Japan;* [d]*Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan;* [e]*Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan*

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Biological context

The human FES (feline sarcoma oncogene) protein is one of two members of a family of non-receptor tyrosine kinases. FES was originally isolated as a retroviral oncogene in avian and feline retroviruses. Genetic analysis identified its cellular homologs, *fes/fps*. The function of FES is still not fully understood. However, it is known to be involved in the growth and differentiation of myeloid hematopoietic cells, vascular endothelial cells and neurons. It is also implicated in the regulation of cytoskeletal rearrangement (Greer, 2002; Takashima et al., 2003).

FES contains multiple domains: Fps/Fes/Fer/CIP4 homology, Src homology 2 (SH2), coiled coil, and tyrosine kinase. Of particular interest is the SH2 domain (residues 450–550), which is implicated in maintaining FES in an inactive state (Takashima et al., 2003).

Methods and experiments

The human FES SH2 domain was produced as a 147-amino-acid recombinant protein with an N-terminal HAT affinity tag and a TEV protease cleavage site. The ^{13}C- and ^{15}N-labeled protein was produced by the *E. coli* cell-free synthesis system (Kigawa et al., 1999). The protein was first adsorbed to a HisTrap HP affinity column (Amersham Biosciences) using 20 mM Tris–HCl (pH 8.0) containing 1 M NaCl and 12 mM imidazole, and eluted with 20 mM Tris–HCl (pH 8.0) containing 500 mM NaCl and 500 mM imidazole. After exchange of buffer to 20 mM Tris–HCl (pH 8.0) containing 1 M NaCl and 15 mM imidazole, the HAT-tag was removed by incubation with the TEV protease at 30 °C for 1 h. To remove the HAT-tag and TEV protease from the reaction mixture, the solution was applied to a HisTrap HP affinity column. The flowthrough fraction was desalted and loaded onto a HiTrap SP column (Amersham Biosciences) equilibrated with 20 mM Tris–HCl (pH 8.0) containing 1 mM EDTA. The flowthrough fraction was applied to a HiTrap Q anion exchange column (Amersham Biosciences) equilibrated with 20 mM Tris–HCl (pH 8.5) containing 1 mM EDTA. Finally, the purified protein was eluted with a gradient of 0–1 M NaCl.

The protein sample used for the NMR measurements comprises 114 amino acid residues. Residues 8–108 constitute the SH2 domain which is surrounded by non-native flanking sequences of residues 1–7 and 109–114 that are related to

*To whom correspondence should be addressed. E-mail: guentert@gsc.riken.jp
**Present address: University of Utah, Department of Biochemistry, Salt Lake City UT 84103-3201, U.S.A.

the expression and purification system. A single sample of approximately 1.2 mM, uniformly ^{13}C and ^{15}N labeled protein was prepared in 20 mM Tris–HCl buffer, pH 7.0, 100 mM NaCl, 1 mM dithiothreitol, 0.02% NaN_3, 10% D_2O (v/v). All NMR measurements were performed at 25 °C on Bruker DRX 600 or, in the case of the NOESY experiments, Bruker AV 800 spectrometers. ^1H, ^{15}N and ^{13}C chemical shifts were referenced relative to the frequency of the ^2H lock resonance of water.

Sequence-specific assignments of the polypeptide backbone resonances were obtained by standard triple resonance techniques (Cavanagh et al., 1996) using 2D [^1H, ^{15}N]-HSQC (Figure 1) and 3D HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH spectra. A 3D HBHA(CO)NH spectrum was used for the assignment of the H^{α} and H^{β} protons. Almost complete ^1H and ^{13}C assignments of the non-aromatic side-chain CH_n moieties, including all prolines, were obtained from analysis of 2D [^1H,^{13}C]-HSQC, 3D (H)CC(CO)NH with 20 ms mixing time, 3D H(CCCO)NH with 20 ms mixing time and 3D HCCH-TOCSY with 14 ms mixing time and 3D HCCH-COSY spectra. Assignments were confirmed using 3D ^{15}N-edited NOESY-HSQC and ^{13}C-edited NOESY-HSQC spectra with 80 ms mixing time. The ^1H and ^{13}C spin systems of the aromatic rings of His, Phe, Trp and Tyr were identified using a 3D HCCH-COSY experiment. Sequence-specific assignments of aromatic side chains were obtained using NOEs between the aromatic protons and the βCH_2 group or the α-proton in a 3D ^{13}C-resolved [^1H,^1H]-NOESY (80 ms mixing time) spectrum.

The programs NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994) were used for spectral processing and data analysis, respectively.

Extent of assignments and data deposition

Excluding the unassigned purification tag residues 1-6, all non-labile ^1H chemical shifts and all backbone amide ^1H chemical shifts except H^N of Asp 56 and H^N of Val 102 could be assigned. All hydrogen-bound ^{15}N resonances are assigned except for N^{ε}/N^{η} of Arg 25, N^{ε}/N^{η} of Arg 60 and N^{ζ} of the 4 Lys residues. All hydrogen-bound ^{13}C resonances and all backbone carbonyl carbon resonances are assigned. The ^1H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 6331.

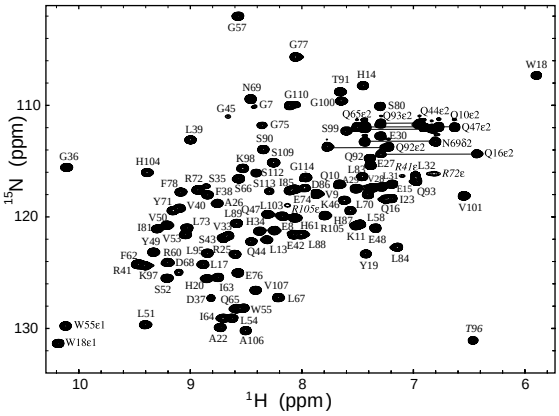

Figure 1. [^1H, ^{15}N]-HSQC spectrum of the SH2 domain of human FES recorded at 600 MHz and 25 °C using 1.2 mM uniformly ^{13}C and ^{15}N labeled protein, 20 mM Tris–HCl, pH 7.0, 100 mM NaCl, 1mM dithiothreitol, 0.02% NaN_3, 10% D_2O (v/v). Folded peaks are labeled in italics and cross-peaks connected by horizontal lines correspond to side chain NH_2 groups of Asn and Gln residues.

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