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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the catalytic domain of guanine nucleotide exchange factor SopE2 from *Salmonella dublin*

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Abstract

Abbreviation: GEF – guanine nucleotide exchange factor.

Biological context

As with other Gram negative pathogens, type III secretion is central to the pathogenesis of *Salmonella enterica*. Type III secretion systems resemble molecular syringes for the injection of multiple bacterial effector proteins into the host cell cytoplasm that modify host cell physiology (Cornelis, 2000). *Salmonella* possesses two type III secretion systems, TTSS1 and TTSS2. TTSS1 is employed in initial invasion of the host cell and in eliciting enteropathogenic responses. TTSS2 is employed in intracellular survival. Among other effects, virulence proteins delivered by TTSS1 cause pronounced membrane ruffling and actin cytoskeleton rearrangements, leading to bacterial internalisation.

Invasion by *Salmonella* depends on the host cell Rho GTPases Cdc42 and Rac1, proteins that regulate diverse signalling pathways including those controlling actin cytoskeleton organisation (Hall and Nobes, 2000). GTPases function as molecular switches that cycle between GTP-bound 'on' and GDP-bound 'off' states. Guanine nucleotide exchange factors (GEFs) facilitate exchange of bound GDP and promote binding of GTP (turn the Rho GTPase switch 'on') (Sprang, 2001).

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Two of the proteins secreted by *Salmonella* TTSS1 are SopE (Wood et al., 1996) and SopE2 (Bakshi et al., 2000). SopE is a potent GEF for both Cdc42 and Rac1 *in vitro* and *in vivo* whereas SopE2 efficiently activates Cdc42 but not Rac1 (Friebel et al., 2001). SopE and SopE2 of *Salmonella typhimurium* are 69% identical. Intracellular activity of SopE induces membrane ruffling and actin cytoskeleton rearrangement similar to that observed upon *Salmonella* invasion (Hardt et al., 1998). *sopE2* appears to be present in all non-typhoidal *Salmonella* lineages, whereas *sopE* is encoded by a bacteriophage that is present in only a few strains.

The recent crystal structure of a SopE-Cdc42 complex indicates that the SopE fold differs from those of eukaryotic GEFs for Rho GTPases (Buchwald et al., 2002). Here we report ^{1}H, ^{13}C and ^{15}N NMR assignments of the catalytic domain of SopE2 towards determination of the solution structure of SopE2 and studies of its dynamics and function.

Methods and experiments

A DNA fragment encoding SopE2(69–240) was amplified by PCR using *S. dublin* chromosomal DNA as a template and cloned into the pGEX-2T vector (Amersham Biosciences). The resulting plasmid was used to transform *E. coli* strain BL21(DE3). ^{15}N-labelled and

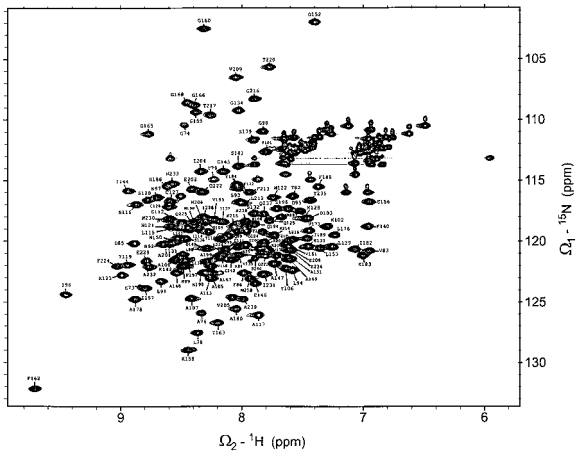

Figure 1. ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-enriched SopE2 catalytic domain (residues 69–240). Assignments are indicated alongside the corresponding signals. Side chain NH_2 groups of Asn and Gln are indicated by lines parallel to the ^{1}H axis. Signals without a residue number could not be assigned.

^{15}N/^{13}C-labelled SopE2 were produced by expression in minimal medium with $^{15}NH_4Cl$ and $^{15}NH_4Cl/^{13}C_6$-D-glucose as the sole nitrogen and nitrogen/carbon sources.

GST-SopE2 was purified using glutathione sepharose 4B resin (Amersham Biosciences) according to the manufacturer's instructions. Human plasma thrombin (Calbiochem) was used to cleave SopE2 from GST whilst bound to the glutathione sepharose 4B resin. SopE2 was subsequently purified further using cation exchange chromatography (Mono S, Amersham Biosciences). The resulting protein contains SopE2 residues 69–240.

NMR samples contained 10 mg of SopE2 in 0.5 ml of 20 mM HEPES, pH 7.0, 50 μM NaN_3 and 5 mM DTT in 95% H_2O/5% D2O. The protein solution was transferred to either Wilmad 535-PP or Shigemi NMR tubes under a nitrogen atmosphere.

NMR data were recorded on a Varian Unity Inova 600 spectrometer at 25 °C. Sequence-specific backbone resonance assignments were made using 3D HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, (HB)CBCACO(CA)HA, HNHA and HBHA (CBCACO)NH data sets. Side chain resonance assignments were made using CCC-TOCSY-NNH, HCC-TOCSY-NNH, HCCH TOCSY and ^{15}N-edited TOCSY (12.1 ms, 12.1 ms, 15.6 ms and 50 ms mixing times). Sequence-specific assignments of aromatic side chains were obtained using ^{1}H-^{1}H 2D DQF COSY, ^{1}H-^{1}H 2D TOCSY (30 ms and 50 ms mixing times), and intra-residue NOEs between the βCH_2 and/or α-proton and the aromatic ring protons in ^{1}H-^{1}H 2D NOESY spectra (100 ms and 175 ms mixing times) and a 3D ^{13}C-edited NOESY spectrum (100 ms mixing time).

All data were processed using NMRPipe (Delaglio et al., 1995) and analysed using Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco). ^{1}H chemical shifts were referenced to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly to DSS (Wishart et al., 1995).

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of the ^{15}N-labelled GEF domain of SopE2 is shown in Figure 1. Backbone N, NH, Cα, Cβ, CO, Hα and Hβ resonances have been assigned for 90% of the residues in SopE2 (69–240). Cα, Cβ, CO, Hα and Hβ resonances, but not N and NH resonances, have been assigned for a further 7% of residues. Complete assignment of side chain ^{13}C resonances has been made for the vast majority of this 97% of residues. For the remainder of the residues, no resonance assignments could be made.

The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-5701.

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