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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 BopE from *Burkholderia pseudomallei*

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Biological context

Burkholderia pseudomallei is the aetiological agent of melioidosis, a severe emerging disease of humans and animals that is endemic in south-east Asia and tropical Australia and that has the potential to spread worldwide (Dance, 2002). Melioidosis has a range of clinical manifestations, including rapidly fatal septicaemia, pneumonia, skin and soft tissue abscesses, and osteomyelitis or septic arthritis. Infection is usually via contaminated soil, dust or water (Brett and Woods, 2000).

The molecular mechanisms of *B. pseudomallei* pathogenesis are not well characterized, although *B. pseudomallei* contains at least three loci encoding putative type III secretion systems (Rainbow et al., 2002). One of these is homologous to the inv/spa/prg type III secretion system (TTSS) of *Salmonella typhimurium* (Stevens et al., 2002). TTSSs are central to the virulence of many Gram negative pathogens, including *Salmonella*, *Shigella*, *Yersinia*, enteropathogenic *E. coli* and the four major genera of plant pathogenic bacteria (Cornelis and van Gijsegem, 2000). 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 to the benefit of the pathogen.

Among the potential effector proteins identified in a recent analysis of the *B. pseudomallei* genome sequence (Stevens et al., 2002), BopE shares sequence homology with the type III-secreted proteins SopE (Wood et al., 1996) and SopE2 (Bakshi et al., 2000) of *Salmonella* (overall 16% sequence identity and 25% identity when the catalytic domains are compared). SopE and SopE2 play an important role in *Salmonella* invasion of non-phagocytic intestinal epithelial cells. SopE is a potent guanine nucleotide exchange factor (GEF) for the mammalian Rho GTPases Cdc42 and Rac1 *in vitro* and *in vivo* whereas SopE2 efficiently activates Cdc42 but not Rac1. SopE also catalyses nucleotide exchange in Rab5, a GTPase involved in intracellular vesicle transport. It has been shown recently that inactivation of *bopE* impairs bacterial entry into HeLa cells indicating that BopE facilitates invasion (Stevens et al., 2003). Consistent with this notion, BopE expressed in eukaryotic cells induced rearrangements in the subcortical actin cytoskeleton, and purified BopE exhibited guanine nucleotide exchange factor activity for Cdc42 and Rac1 *in vitro* (Stevens et al., 2003). Here we report backbone and side chain ^{1}H, ^{13}C and ^{15}N NMR assignments of the catalytic domain of BopE.

Methods and experiments

A DNA fragment encoding BopE residues 78–261 was amplified by PCR using *B. pseudomallei* 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 $^{15}N/^{13}C$-labelled $BopE_{78-261}$ 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-$BopE_{78-261}$ was purified using glutathione sepharose 4B resin (Amersham Biosciences) according to the manufacturer's instructions. Human plasma thrombin (Calbiochem) was used to cleave

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$BopE_{78-261}$ from GST whilst bound to the glutathione sepharose 4B resin. $BopE_{78-261}$ was subsequently purified further using anion exchange chromatography (Mono Q, Amersham Biosciences). The final protein contains Gly and Ser from the pGEX-2T vector at the N-terminus followed by $BopE_{78-261}$.

NMR samples contained 10 mg of $BopE_{78-261}$ in 0.5 ml of 20 mM potassium phosphate, pH 5.5, 50 mM NaCl, 10 mM DTT, 1 mM EDTA, 1mM benzamidine and 50 μM NaN_3 in 95% H2O / 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, HN(CA)CO, (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 TOCSY (28 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 simultaneous ^{15}N/^{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. ^{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 BopE is shown in Figure 1. Backbone N, NH, Cα, Cβ, CO, Hα and Hβ resonances have been assigned for all but one of the residues in $BopE_{78-261}$. Complete assignment of side chain ^{13}C and ^{1}H resonances has been made for approximately 90% of these residues.

The ^{1}H, ^{13}C and ^{15}N chemical shifts of $BopE_{78-261}$ have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-5974.

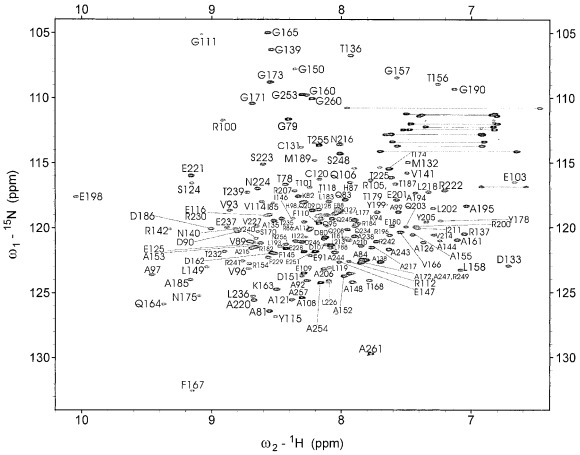

Figure 1. ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-enriched BopE catalytic domain (residues 78-261). Assignments are indicated alongside the corresponding signals. Side chain NH_2 groups of Asn and Gln are indicated by lines parallel to the ^{1}H axis.

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