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ARTICLE

NMR assignment and secondary structure of the STAS domain of Rv1739c, a putative sulfate transporter of *Mycobacterium tuberculosis*

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Abstract We report $^{1}H^{N}$, ^{15}N, and ^{13}C resonance assignments for the 15.6 kDa STAS domain of the putative sulfate transporter of *Mycobacterium tuberculosis*, Rv1739c, using heteronuclear, multidimensional NMR spectroscopy. Rv1739c is a SulP anion permease, related in structure to the SLC26 gene family of metazoan anion exchangers and anion channels.

Keywords SLC26 · Sulfate · SulP · SpoIIAA · Anti-sigma factor antagonist

Biological context

The 10 human SLC26 genes encode anion exchangers and anion channels of homologous sequence, with cytoplasmic amino- and carboxy-termini linked by polytopic membrane domains that traverse the lipid bilayer an estimated 10–12 times (Dorwart et al. 2008a, b). Among this family of genes there are four in which homozygous or compound heterozygous mutations cause Mendelian recessive congenital human and mouse diseases of chondrodysplasia (SLC26A2), chloride diarrhea (SLC26A3), deafness (SLC26A4 and SLC26A5), and late-onset goiter (SLC26A4 only in humans). Engineered deletions of mouse SLC26 genes also lead to hyperoxaluric urolithiasis (Slc26a6), altered regulation of gastrointestinal NaCl absorption and HCO_3 secretion (Slc26a6) distal renal tubular acidosis (Slc26a7), male infertility (Slc26a8), and severe to moderate gastric achlorhydria (Slc26a9 and Slc26a7, respectively). The SLC26 carboxy-terminal tails share "sulfate transporter and anti-sigma-factor-antagonist" (STAS) domains (Aravind and Koonin 2000) that have also been identified in the related SulP sulfate/anion transporters of plants, yeast, and bacteria (Felce and Saier 2004). The prototype STAS domain protein is the SpoIIAA anti-sigma factor antagonist of *B. subtilis*. Starvation-induced sporulation of *B. subtilis* requires induction of a transcriptional program that is dependent upon RNA polymerase activation by sigma factor σ^{F}, the first of several, sequentially activated sigma factor promoter specificity determinants of RNA polymerase. Truncations and mutations in the STAS domains of mammalian SLC26 transporters also impair the function and/or surface expression of these transporters (Chernova et al. 2003; Dorwart et al. 2008a, b). The STAS domains of SLC26A3 and SLC26A6 interact with the R domain of cystic fibrosis transmembrane regulator, producing reciprocal activation in vitro (Dorwart et al. 2008a, b).

Crystal and NMR structures of phosphorylated and unphosphorylated SpoIIAA have been reported (Seavers et al. 2001 and Clarkson et al. 2003). The structure of the putative anti-sigma factor antagonist TM1442 from *Thermotoga maritima* has also been solved, first by X-ray

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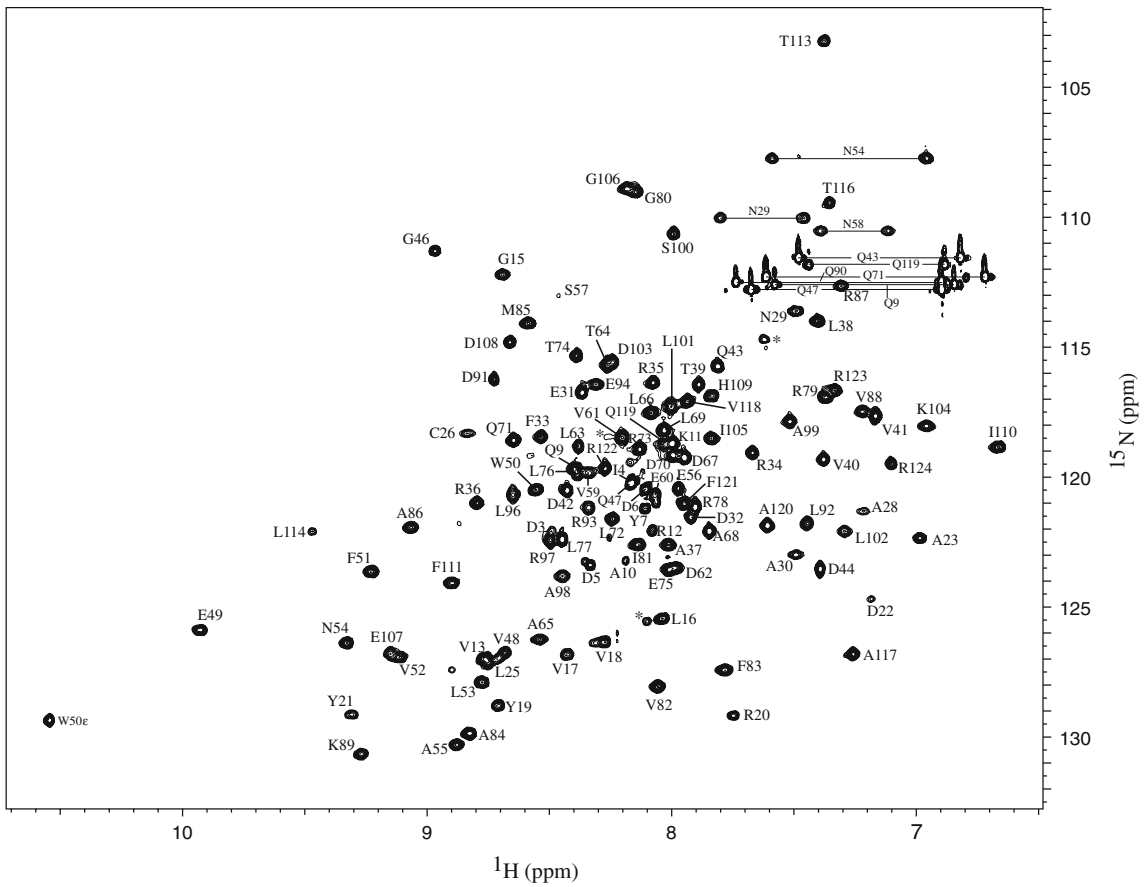

Fig. 1 2D ^{1}H–^{15}N HSQC spectrum illustrating the assigned residues from a 0.7 mM STAS domain sample in 50 mM Na-phosphate, 275 mM NaCl, pH 7.2. This spectrum was recorded on a Bruker Avance 600 MHz spectrometer at 298 K. The assignments shown are annotated using the one letter amino acid code followed by the sequence number of that residue. Side-chain amide protons of Trp, Asn and Gln are also illustrated. Horizontal lines represent the connectivity between the two side-chain amide protons of each Asn and Gln residue. The crosspeaks marked with '*' represent His residues from His-tag present at C-terminus of STAS domain

diffraction (Lee et al. 2004) and subsequently in unphosphorylated and phosphorylated states by NMR spectroscopy (Etezady-Esfarjani et al. 2006). The structures of STAS domains in the C-terminal cytoplasmic tails of anion transporters remain unknown, although a preliminary, high resolution ^{1}H–^{15}N TROSY-HSQC NMR spectrum of the human SLC26A3 STAS domain has been recently reported (Dorwart et al. 2008a, b), Here we provide the backbone and side-chain assignments for the STAS domain of the *Mycobacterium tuberculosis* SulP polypeptide Rv1739c, a putative sulfate transporter (Zolotarev et al. 2008).

Methods and experiments

Rv1739c SulP DNA (Zolotarev et al. 2008) encoding the C-terminal STAS domain, which extends from amino acid 437–560, was PCR-cloned with a C-terminal 6His tag, subcloned into the expression vector pETBlue-1, and transformed into Tuner DE3pLacI cells (Novagen). STAS-6His proteins uniformly labeled with ^{15}N or with $^{13}C/^{15}N$ were prepared by growing *E. coli* cells harboring pET-Blue1-STAS-6His in minimal media containing $^{15}NH_4Cl$ (1 g/l)/$^{13}C_6$-glucose (2 g/l), or $^{15}NH_4Cl$ (1 g/l) (Cambridge Isotope Laboratories) supplemented with Bio-Express 1000 (Cambridge Isotope Laboratories). After protein induction by IPTG, the bacteria were lysed with BugBuster (Novagen). Inclusion bodies were pelleted and solubilized for 1-h with rotation in 4 M guanidine HCl, pH 8.0, containing 50 mM Na phosphate, 300 mM NaCl, 10 mM imidazole and 2 mM β-mercaptoethanol. After removal of insoluble cell debris by centrifugation, the STAS domain-containing supernatant was mixed overnight with Ni-NTA beads (Qiagen) and subjected to an on-column refolding procedure modified using a published protocol (Oganesyan et al. 2004). The bound beads were washed sequentially with 10 volumes of the following several buffers, all at pH 8.0 and containing (in mM): (1) 4,000 guanidine HCl, 50 Na phosphate, 300 NaCl, 20 imidazole, 2 β-mercaptoethanol;

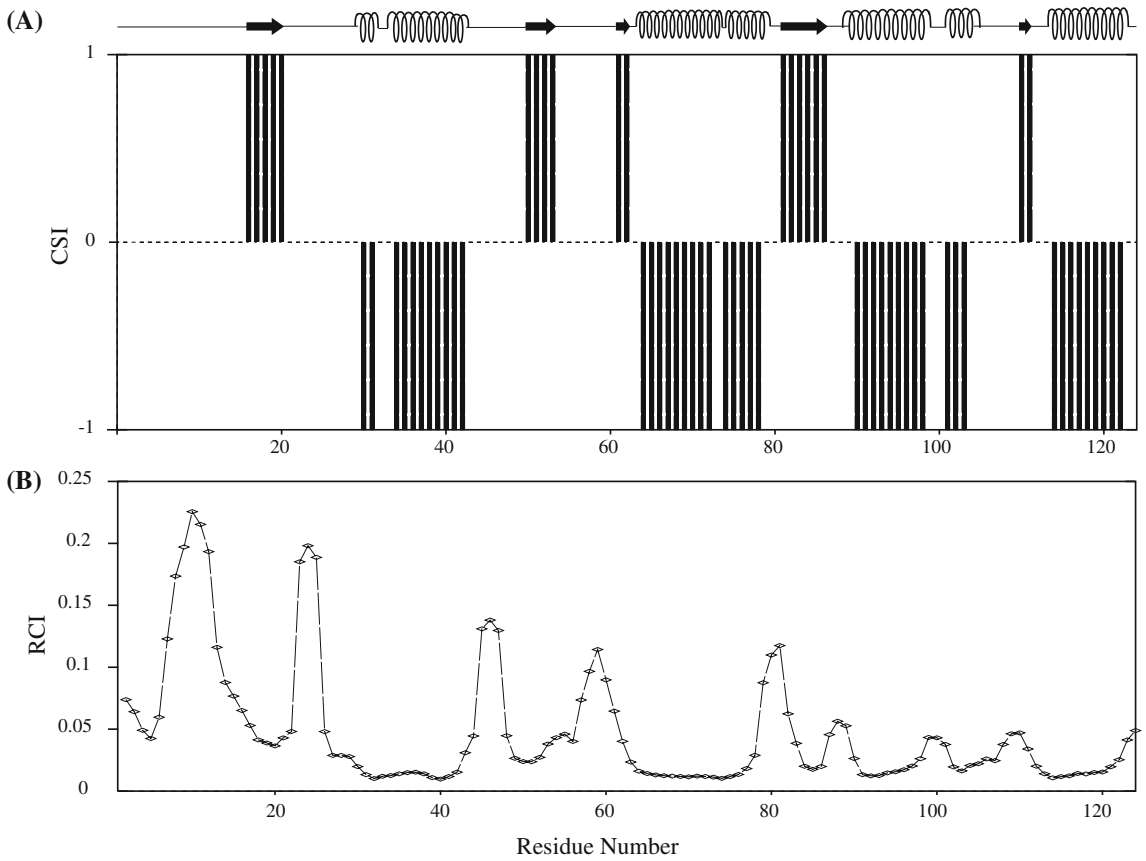

Fig. 2 **a** The consensus chemical shift index ($^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$) versus residue number plot of the STAS domain showing β-strands (CSI value of +1) and α-helices (CSI value of −1); for clarity these regions are indicated by arrows and helices, on the *top*. **b** The random coil index (based on $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$) versus residue number plot of the STAS domain. The flexible regions are shown by residues adopting high RCI values

(2) 0.1% Triton ×100, 100 mM NaCl, 20 mM Tris–HCl; (3) 50 Na phosphate, 300 NaCl, 20 imidazole; (4) 5 β-cyclodextrin, 100 NaCl, 20 Tris–HCl; (5) 500 NaCl, 20 Tris–HCl; (6) 300 NaCl, 50 Na phosphate. Nominally refolded STAS domain was then eluted from the beads with solution containing 250 mM imidazole in 300 mM NaCl, 50 mM Na phosphate, pH 7.2. Eluates were subjected to seven cycles of 10-fold dilution followed by spin concentration with Amicon Ultra-15 spin filters to remove imidazole and to concentrate STAS-6His domain polypeptide. The purified protein as detected by Coommassie Blue Staining after SDS-PAGE or PFO-PAGE was a single band of ~15 kDa with apparent purity of >96%.

NMR samples of $^{13}C/^{15}N$- and ^{15}N-STAS-6His (0.7–0.8 mM) were prepared in a 92% H_2O/8% D_2O solvent composition containing 50 mM Na phosphate, 275 mM NaCl, 2 mM DTT-d_{10}, 0.25 mM 2,2-dimethyl-2-silapentanesulfonic acid (DSS) as internal standard, and 0.05% (w/v) NaN_3 to avoid any unwanted bacterial growth over time. All NMR experiments were performed at 298 K on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm triple resonance PFG (z-axis) probe. These data were acquired in the gradient-selected sensitivity-enhanced mode. Backbone and ^{13}C side-chain assignments were carried out using triple resonance experiments of HNCACB, HN(CO)CACB, HNCA, HN(CO)CA, C(CCO)NH, HNCO, and HN(CA)CO, and 3D ^{15}N-edited NOESY-HSQC (τ_m = 120 ms) (Cavanagh et al. 1996). These NMR data were processed on an Intel PC workstation running Redhat Linux 7.1 using NMRPipe/NMRDraw (Delaglio et al. 1995). The ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced to the internal standard DSS using IUPAC-IUB recommended protocols (http://www.bmrb.wisc.edu/ref_info/cshift.html). All NMR spectra were visualized and analyzed using ANSIG (Kraulis 1989).

Assignments and data deposition

The assignment of $^{1}H^{N}$, ^{13}C, and ^{15}N chemical shifts of the STAS domain of Rv1739c of *Mycobacterium tuberculosis* have been deposited into BMRB, accession number 16052.

Figure 1 is the 2D ^{1}H–^{15}N HSQC spectrum identifying the $^{1}H^{N}$, ^{15}N assignment of 112 out of 119 non-proline

residues. $^{1}H^{N}$, ^{15}N cross-peaks belonging to residues M1, H2, F27, N58, Q90, S95, and M112 are not visualized in our 2D ^{1}H–^{15}N HSQC, presumably due to the exchange of these $^{1}H^{N}$ protons with the bulk solvent. However, using resonance assignment information from our triple resonance spectra, the other resonances for these amino acids have been assigned with the exception of the first residue, M1. Five proline residues were also assigned based on identification of their ^{13}C chemical shifts. Side-chain ^{1}H–^{15}N correlations for all asparagine (ASN) and glutamine (GLN) residues have been assigned, as illustrated in the Fig. 1. In total, assignments were made for 95.5% of backbone resonances, including $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, and $^{13}C'$. Side-chain ^{13}C assignments were achieved for 224 out of a possible 269 aliphatic carbon resonances (including 95.9% of $^{13}C^{\beta}$ assignments).

A chemical shift index (CSI) (Wishart and Sykes 1994) consensus for the $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ atoms suggest that the STAS domain comprises a mixed distribution of α helix and β sheet secondary structure (Fig. 2). The random coil index (Berjanskii and Wishart 2008), which uses $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ chemical shift assignment data identified that the residues undetected in the 2D ^{1}H–^{15}N HSQC belong to unstructured flexible loops within the STAS domain, with the exceptions of S95 and Q90 (Fig. 2). ClustalW2 sequence alignment analysis (http://www.ebi.ac.uk/Tools/clustalw2/index.html) shows alignment of the regions contributing to STAS domain α-helices and β-strands in Rv1739c with those of SPOIIAA (PDB ID 1AUZ) and TM1422 (PDB ID 1SBO). Based on average tertiary structures of SPOIIAA and TM1422, the respective values for percent secondary structure present in the Rv1739c STAS domain, SPOIIAA, and TM1422 are 55.6, 53.4, and 56.3. Efforts are underway to collect additional NMR data needed to complete our ^{1}H assignments and to calculate the three-dimensional solution structure of the Rv1739c STAS domain.

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