NMR assignments of the GacS histidine-kinase periplasmic detection domain from Pseudomonas aeruginosa PAO1

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NMR assignments of the GacS histidine-kinase periplasmic detection domain from *Pseudomonas aeruginosa* PAO1

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Abstract *Pseudomonas aeruginosa* is a highly adaptable opportunistic pathogen. It can infect vulnerable patients such as those with cystic fibrosis or hospitalized in intensive care units where it is responsible for both acute and chronic infection. The switch between these infections is controlled by a complex regulatory system involving the central GacS/GacA two-component system that activates the production of two small non-coding RNAs. GacS is a histidine kinase harboring one periplasmic detection domain, two inner-membrane helices and three H1/D1/H2 cytoplasmic domains. By detecting a yet unknown signal, the GacS histidine-kinase periplasmic detection domain (GacSp) is predicted to play a key role in activating the GacS/GacA pathway. Here, we present the chemical shift assignment of 96 % of backbone atoms (HN, N, C, Cα, Cβ and Hα), 88 % aliphatic hydrogen atoms and 90 % of aliphatic carbon atoms of this domain. The NMR-chemical shift data, on the basis of Talos server secondary structure predictions, reveal that GacSp consists of 3 β-strands, 3 α-helices and a major loop devoid of secondary structures.

Keywords *Pseudomonas* · Two-component system · GacS · Protein · NMR

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Biological context

Pseudomonas aeruginosa (PA) is a major opportunistic pathogen, responsible for nosocomial infections in immunocompromised patients. It can be involved in both acute and chronic infections (Gooderham and Hancock 2009). During acute infection, PA activates genes responsible for bacterial motility and excessive production of toxins that will be liberated in the extracellular medium or directly injected in host cells via type III secretion system. In contrast, chronic infections are characterized by formation of persistent bacterial communities enclosed by polysaccharide-rich matrix (biofilm) that provides high level of resistance against host innate immune response and antibacterial treatments (Furukawa et al. 2006). The switch between chronic and acute infection is regulated by a complex regulatory network involving two-component systems (TCSs), di-GMPc level and quorum sensing (QS). In this regard, environmental stresses and unknown signals detected by PA trigger expression of genes responsible for one infection type or another (Rasamiravaka et al. 2015).

TCSs are ubiquitous regulatory systems found in both prokaryotes and eukaryotes but absent from mammalians. They play essential role for the adaptation and survival of the organism by modulating cellular functions in response to environmental changes. TCSs comprise classically a membrane embedded histidine kinase (HK) sensor, which acts as a dimer, and a cognate response regulator (RR). Detection of environmental stimuli by the HK sensor leads to the autophosphorylation of its histidine kinase domains. The phosphate group is afterward transmitted to the cognate RR. Consequently, the activated RR triggers expression of different target genes (Mitrophanov and Groisman 2008). Comparing to 30 HKs and 34 RRs encoded by

Escherichia. coli, PA uses 10 % of its genes to encode for 64 HK and 72 RR, which reflects the adaptation capacities of this bacterium (Rodrigue et al. 2000).

In PA, the GacS/GacA TCS plays a key role in the transition between acute and chronic infection that is antagonistically modulated by the two other hybrid histidine kinase sensors, LadS and RetS (Goodman et al. 2004; Ventre et al. 2006). The activation of GacS/GacA pathway induces expression of non-coding small RNA (sRNAs), RsmY and RsmZ (Brencic et al. 2009). Subsequently, an increased level of intracellular sRNAs leads to sequestrate the RNA-binding protein, RsmA. RsmA is a translational repressor that binds to mRNAs and modulates expression of more than 500 genes, including QS and T6SS gene. The GacS/GacA TCS positively regulates expression of genes required for biofilm formation, like *psl* and *pel* operons responsible for the production of Psl and Pel expolysaccharides that represent major components of the biofilm matrix. Conversely, it down-regulates expression of T3SS and motility factors (flagellum) (Bordi et al. 2010; Goodman et al. 2004).

GacS is a histidine kinase harboring one periplasmic detection domain, two inner-membrane helices and three H1/D1/H2 cytoplasmic domains. Compared to a classical HK, the GacS HK is an unorthodox sensor that possesses a second receiver domain (D1) and an alternative histidine-phosphotransfer domain (H2). In this case, the phosphorelay mechanism requires a four-step pathway. Autophosphorylation of a conserved His residue on the H1 domain initiates a series of phosphotransfer reactions involving HisH1 → AspD1 → HisH2. Afterward, the cognate response regulator GacA is activated by receiving a phosphate group from the GacS H2 domain (Chambonnier et al. 2016) In this system, GacSp is predicted to play a decisive sensing role. Whereas, post-binding conformational changes might engender structural rearrangements in the transmembrane helices leading to the activation of the H1 domain phosphorylation (Heeb and Haas 2001). Moreover, the nature of the signal that binds to GacSp is still unknown. To date, there is no structural information for this domain which limits our understanding regarding its mode of action. Here we report the backbone and side-chain resonance chemical shift assignments (^{1}H, ^{13}C, ^{15}N) and identify the secondary structure elements of the periplasmic detection domain of the GacS HK sensor from PA.

Expression and purification of GacSp

The DNA sequence, coding for the periplasmic domain of GacS (38–164), was cloned into pET21a plasmid containing an oligohistidine tag (MGHHHHHHSSGVDLGTENLYFQS). *E. coli* BL21 (DE3) cells (Novagen) harboring pET21a-*gacSp* were cultured overnight at 37 °C in 100 mL of LB medium containing 50 µg/mL kanamycin and 34 µg/mL chloramphenicol. The cells were harvested by centrifugation and resuspended in 1 L of M9 growth medium containing 4 g/L (^{13}C)-D-glucose, 1 g/L (^{15}N)-NH_4Cl (Cambridge isotope Laboratories), 50 µg/mL kanamycin and 34 µg/mL chloramphenicol. The culture was grown at 37 °C to an OD_{600} of 0.6. IPTG (0.2 mM) was added and the cells were incubated overnight at 20 °C for GacSp overproduction. Cells were harvested by centrifugation (5000 g, 15 min, 10 °C) and the pellet was resuspended in 50 mL of lysis buffer (50 mM Tris/HCl, 300 mM NaCl, 10 mM imidazole, 5 % glycerol, 1 tablet of SIGMA-FAST™ EDTA-free protease inhibitor cocktail and lysosyme pH 8) and disrupted by sonication on ice after the addition of 20 mM $MgSO_4$ and DNase. The lysate was clarified by centrifugation (13,000×*g*, 40 min, 4 °C) and the supernatant was applied onto nickel-chelate affinity resin column (5 mL) using an ÄktaXpress (GE-Healthcare). The column was washed with five volumes of 50 mM Tris–HCl, 300 mM NaCl, and 40 mM imidazole pH 8 and the protein was eluted with 50 mM Tris–HCl, 300 mM NaCl, and 250 mM imidazole pH 8. GacSp was concentrated and further purified by gel filtration (GF) on a Superdex75 16/60 column (GE-Healthcare) equilibrated with 50 mM sodium phosaphate pH 7 and 150 mM NaCl (GF buffer). Pure fractions of GacSp, as analyzed by SDS-PAGE electrophoresis, were pooled and concentrated to 12.5 mg/mL using a 10 kDa cut-off ultracentrifugation membrane (Thermoscientific).

NMR spectroscopy

NMR data were collected at 298 K on a 0.8 mM sample of ^{13}C,^{15}N-labeled GacSp in GF buffer containing 10 % D_2O. All the NMR experiments required for backbone and side-chain assignment were recorded on Bruker Avance III 600 MHz spectrometer (IMM NMR platform, Marseille) and on a 950 MHz Bruker Avance III HD spectrometer (IR-RMN, Grenoble), respectively. Both spectrometers were equipped with a TCI cryoprobe. Data were processed using Topspin 2.1 (Brucker) and analysed by using the program CARA (keller 2004). Backbone chemical shifts were assigned using HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH, HNCO and HN(CA)CO. These values were used to calculate backbone torsion angles (Φ and ψ) using TALOS + server (Shen et al. 2009). Aliphatic side chain carbons were identified using HNHA, HCCH-TOCSY, 2D HSQC-TOCSY, ^{15}N-HSQC-TOCSY, ^{15}N-HSQC-NOESY. Aromatic side chain resonances were assigned using the 2D HSQC-NOESY and the three-dimensional ^{13}C-HSQC-NOESY spectra.

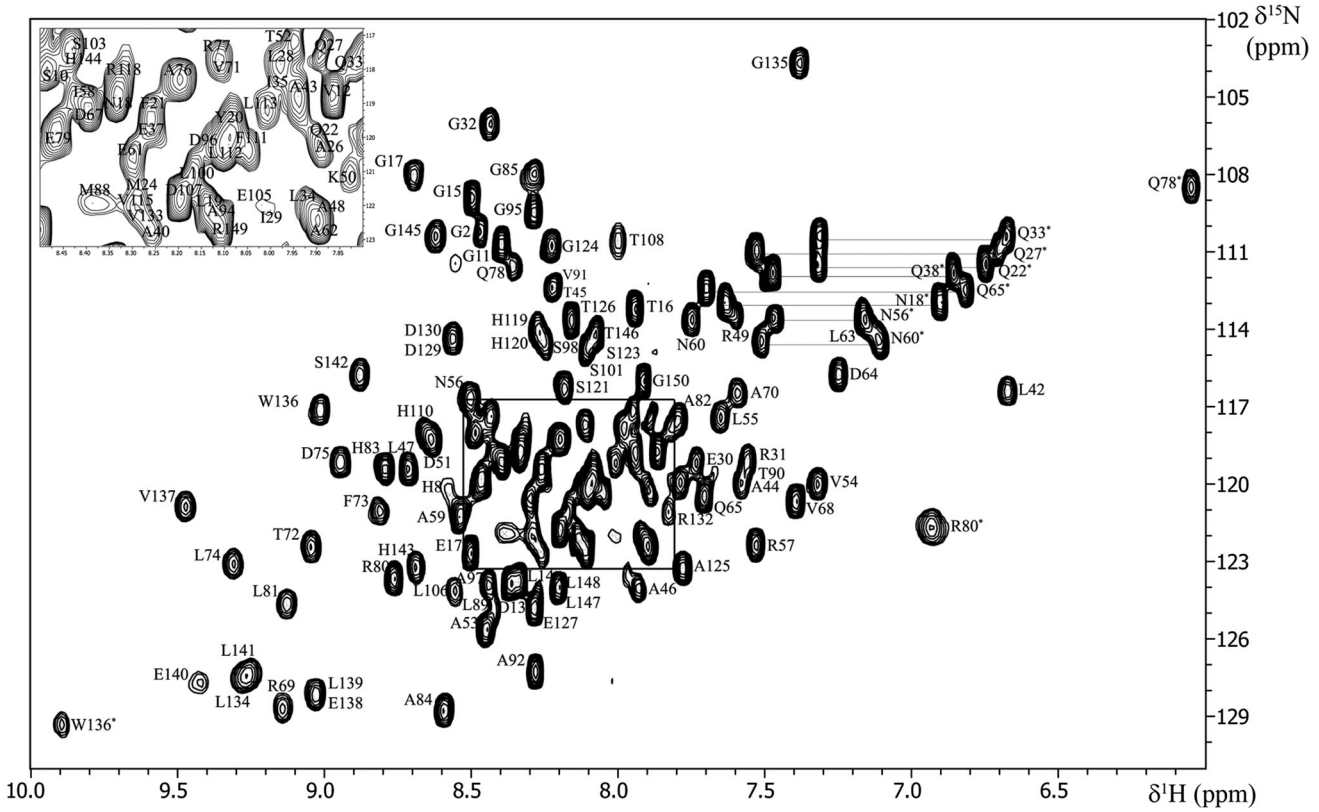

Fig. 1 $^{1}H,^{15}N$-HSQC spectrum of GacSp in 150 mM NaCl, 50 mM KPO_4 buffer pH7, 10 % D_2O, at 298 K on a Bruker Avance III 600 MHz spectrometer. The backbone $^{1}H,^{15}N$ correlations are labeled according to the sequence. Side chain amine resonances are indicated with *star*. Side chain resonances of Gln and Asn residues are connected by *horizontal lines*

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                 10        20        30        40        50        60        70        80
                           α1                            α2                  β1
MGHHHHHHSS GVDLGTENLY FQSMRAQLIE RGQLIAEQLA PLAATALARK DTAVLNRIAN EALDQPDVRA VTFLDARQER
XXXXXXXXLL LLLLLLLLHH LLHHHHHHHH HHHHHHXXXL HHHHHHHHLL LHHHHHHHHH HHHLLLLLEE EEEELLLLLL
0000000099 8876886043 5837999999 9999950005 6889999638 6899999999 9952899669 9998454665

                 90       100       110       120       130       140       150
       β2                  α4                                    β3
LAHAGPSMLT VAPAGDASHL SMSTELDTTH FLLPVLGRHH SLSGATEPDD ERVLGWVELE LSHHGTLLRG
EEEELLLLLL LLLLLLLHHH LLLLLLLLLL LLLLLELLLL HLLLLLLLHH HLEEEEEEEE EELLLLLLLL
5996897767 7988885686 1158888998 5488062784 5469999826 4227899999 9568971030
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Fig. 2 GacSp secondary structure predictions and the confidence scores (0–10) as derived from a Talos+ analysis (H for α-helix, E for β-strand an L for loop)

Assignments and data deposition

The $^{1}H,^{15}N$ HSQC spectrum of GacSp, recorded at 298 K, contains 153 resonances of which 18 correspond to amine groups of glutamine and asparagine and seven correspond to NHε group of arginine side chains (Fig. 1). The first nine residues of the His-tag, except G2 and H8, and S23, R25, S87, H99 and L116 were not assigned. In total, greater than 96 % of backbone atoms (HN, N, C, Cα, Cβ and Hα), 88 % aliphatic hydrogen atoms and 90 % of aliphatic carbons atoms were assigned. Roughly 50 % of the aromatic side chains atoms were attributed, probably due to unfavourable exchange dynamics and GacSp unstable conformation.

The results of the secondary structure prediction, carried out on TALOS + server, are shown in (Fig. 2). GacS is predicted to harbor three N-terminal α-helices (α1, α2 and α3) followed by two β-strands (β1, β2), one α-helix (α4) and a β-strand (β3). A major loop (32 residues) is predicted to link α4 and β3. Chemical shift assignments for GacSp have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 26840.

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