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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances to the sensory domain of the membraneous two-component fumarate sensor (histidine protein kinase) DcuS of *Escherichia coli*

Tatjana N. Parac[a,*], Boris Coligaev[a,*], Evelyne Zientz[b], Gottfried Unden[b], Wolfgang Peti[a,*] & Christian Griesinger[a,**]
[a]*Max-Planck Institute for Biophysical Chemistry, Am Faßberg 11, D-37077 Göttingen, Germany;* [b]*Institut für Mikrobiologie und Weinforschung, Universität Mainz, Becherweg 15, D-55099 Mainz, Germany*

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Biological context

In bacteria various types of signal transduction proteins are used for the perception of environmental signals (Aizawa et al., 2000). Most of the sensors receive the signal at the periplasmic side of the membrane. By transferring the signal across the membrane, it is converted into a cellular signal, which is used for regulation of transcription, enzyme activity, or flagellar rotation. Two-component regulatory systems represent the most frequent systems of this type for transmembrane signalling in bacteria. They consist of a membraneous sensory histidine protein kinase and a cytoplasmic response regulator. Signal transfer between the sensor and the response regulator is effected by protein phosphorylation.

In the facultative anaerobic bacterium *Escherichia coli* the switch from aerobic to anaerobic metabolism is regulated at the transcriptional level in response to the electron acceptors O_2, nitrate, and fumarate. The expression of the genes of anaerobic fumarate respiration, including fumarate reductase, an anaerobic C_4-dicarboxylate (fumarate) carrier, and of fumarase B, is regulated by C_4-dicarboxylates and the two-component regulatory system DcuSR (dicarboxylate uptake) (Zientz et al., 1998; Golby et al., 1999). The DcuSR system consists of the sensor DcuS located in the cytoplasmic membrane, and of the cytoplasmic response regulator DcuR. DcuS contains two transmembrane helices, one periplasmic domain, and the C-terminal kinase and transmitter domain. The kinase domain extends into the cytoplasm of *E. coli* and catalyses autophosphorylation of a conserved histidine residue of the protein. Its activity is controlled by the external and possibly other signals. The periplasmic domain of about 140 amino acid residues is enclosed by the transmembrane helices. This domain binds fumarate and other C_4-dicarboxylates (Zientz et al., 1998). Due to the periplasmic site for signal reception, the signal has to be transferred across the membrane from the sensory to the kinase and transmitter domain. From two-component sensors only the structure of the cytoplasmic CheA histidine kinase, which is involved in chemotaxis regulation (Bilwes et al., 1999), and of the cytoplasmic kinase domain of the osmosensor EnvZ (Tanaka et al., 1998) are known. Thus, for the process of transmembrane signalling no detailed structural information is available. To get an insight into the conformational changes involved in the signal transfer across the membrane, the structure of the periplasmic domain of the DcuSR fumarate sensory system was analysed by NMR spectroscopy. The ^{1}H,^{15}N-HSQC with annotated assignments is shown in Figure 1.

Methods and experiments

The periplasmic domain (amino acid residues 45 to 180) of DcuS was overproduced in *E. coli* strain BL21DE3 carrying the $DcuS_{45-180}$ expression plasmid pMW145. The plasmid was derived from pET28a with the codons for amino acid residues 45 to 180 of *dcuS* cloned in a frame behind the sequence for

*These authors contributed equally.
**To whom correspondence should be addressed at the Max-Planck Institute in Göttingen. E-mail: cigr@mpibpc.mpg.de

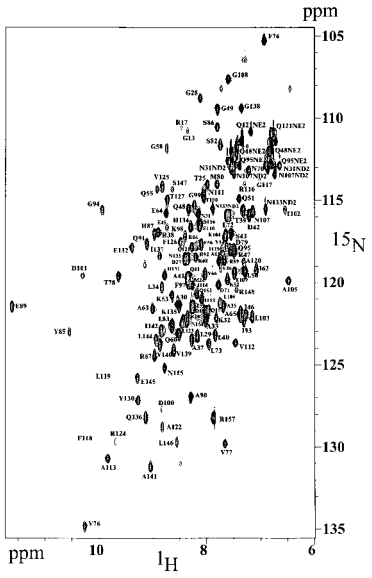

Figure 1. Annotated $^{1}H,^{15}N$-HSQC spectrum of DucS recorded at 303 K and a ^{1}H frequency of 600 MHz. Sequential assignments are indicated with the one-letter amino acid code and the residue number.

the T7 promotor and an N-terminal His_6-tag. The induced strain BL21DE3(pMW145) was disrupted by a French Press, and the $DcuS_{45-180}$ protein was isolated from the cell homogenate by chromatography on a Ni^{2+}-NTA column. After elution, dialysis, and concentration by ultrafiltration, $DcuS_{45-180}$ (20 mg protein/ml) was either stored at $-80\,^{\circ}C$ or directly used for NMR spectroscopy.

About 1 mM ^{15}N and $^{15}N,^{13}C$ labeled DcuS were used for measurement in a 5 mm microcell Shigemi tube (300 µl). The protein sample was at pH 6.5, 50 mM sodium phosphate buffer, 200 mM NaCl, 0.8 mM CHAPS, 50 mM Glycin, 50 pM Pefabloc SC (Fluka AG, Buchs, Switzerland), 0.01% NaN_3 and H_2O/D_2O 90/10. The following experiments were carried out: ^{15}N labeled sample: 2D $^{1}H,^{15}N$-HSQC, 3D NOESY-HSQC (150 ms mixing time), 3D TOCSY-HSQC (60 ms mixing time), 3D HSQC-NOESY-HSQC (100 ms mixing time); $^{15}N,^{13}C$ labeled sample: 3D HNCO, 3D CBCA(CO)NH, 3D HNCACB, 3D CC(CO)NH, 3D HBHACONH, 3D HCCH-COSY and 3D HCCH-adiabatic TOCSY (Peti et al., 2000). Pulsed field gradient versions of the experiments, with water flip-back pulses and sensitivity enhancement (Sattler et al. (1999) and references cited therein) were recorded on a Bruker DRX 600 and Bruker DRX 800 (TXI HCN z-grad) at 303 K (Bruker Analytic GmbH, Rheinstetten, Germany). Fourier transformation, mirror image linear prediction and data analysis were carried out using the programs XWINNMR 2.6 (Bruker) and XEASY (Bartels et al., 1995). Resonance positions were referenced using internal DSS to obtain ^{1}H, ^{13}C and ^{15}N chemical shifts as described in Wishart et al. (1995). The annotated $^{1}H,^{15}N$-HSQC is shown in Figure 1.

Extent of assignments and data deposition

$^{1}H,^{15}N,^{13}C$ backbone resonances of 134 out of 151 non-proline residues could be assigned (the first 20 amino acids belong to the His_6-tag and a linker). The ^{1}H and ^{13}C resonances of all 6 prolines could be assigned. The side chain $H_\alpha,H_\beta,C_\alpha,C_\beta$ resonances could be assigned for 136 of the 137 residues. Additional side-chain proton chemical shifts were identified in a TOCSY-HSQC, the NOESY-HSQC and the HCCH-COSY and HCCH-TOCSY spectra. The chemical shift values of proton, nitrogen and carbon resonances were deposited in the BioMagResBank in Madison, WI, U.S.A. (accession number BMRB-4821).

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