Journal of Biomolecular NMR, **20:** 191–192, 2001.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N sequence-specific resonance assignment of the PSCD4 domain of diatom cell wall protein pleuralin-1

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Received 7 March 2001; Accepted 6 April 2001

Key words: diatom cell wall, NMR assignments, pleuralin, PSCD

Biological context

Pleuralins (formerly HEPs), a previously described diatom protein family (Kröger et al., 1997), are involved in the cell cycle dependent morphogenesis of the silica cell wall consisting of two overlapping half-shells (thecae, Kröger and Wetherbee, 2000). They are located at the inner surface of the terminal elements (pleural bands) of the epitheca. All members of the pleuralin family contain a proline-rich domain followed by 3–5 conserved so-called PSCD domains of 87 or 89 amino acid residues, respectively, rich in proline (22%), serine (11%), cysteine (11%), and aspartate (9%). They contain 10 cysteine residues at exactly the same positions forming disulphide bridges and show 73–91% sequence identity to each other. Pleuralins are tightly incorporated within the silica scaffold, imperishable to EDTA and SDS treatment, but extractable with anhydrous hydrogen fluoride (HF). It seems, therefore, likely that they are connected to the cell wall by covalent linkage to the silanol groups (SiOH) of the silica surface by Si–O–C bonds or by crosslinking with HF-labile glycoside bonds (Kröger et al., 1997). Possible posttranslational modifications have not been analysed so far. In this study we present sequence-specific assignment of the NMR signals of a fragment of pleuralin-1 (formerly HEP200; amino acids 366–468) of *Cylindrotheca fusiformis* (Kröger et al., 1997) which encloses the PSCD4 domain. Pleuralin-1 does not exhibit sequence similarity to any protein in the common data banks. Structure determination of this protein is, therefore, of special interest.

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Methods and experiments

A gene fragment encoding amino acids 366–468 of pleuralin-1 was amplified by PCR from a cloned genomic DNA fragment of *Cylindrotheca fusiformis* (Kröger et al., 1997). A sense primer was used that introduced in frame the coding sequence for the nonapeptide SYYHHHHHH at the 5′-end of the PCR product. To exchange the unfavourable codons for R410 and L416 (amino acid numbering is according to the pleuralin-1 sequence described by Kröger et al., 1997) against codons that are more frequently used in *E. coli* (CGC for R410, TTA for L416), the gene SOEing technique (Horton et al., 1989) was applied using primers bearing appropriate point mutations. The PCR product was cloned into StuI/HindIII digested plasmid pRbi-DsbC (this plasmid was constructed analogous to pRbi-PDI (Wunderlich and Glockshuber, 1993) but PDI was replaced by DsbC from *E. coli*). This cloning strategy yields expression plasmid pHis$_6$PSCD4-DsbC, which drives the coexpression and cosecretion of His$_6$PSCD4 and DsbC into the *E. coli* periplasm.

Cells of *E. coli* BL21(DE3) harbouring plasmid pHis$_6$PSCD4-DsbC were grown in M9 medium (Sambrook et al., 1989) supplemented with a mineral solution and 100 mg/ml Carbenicillin. Cells were grown to $OD_{517} = 1.0$ and induced with 1 mM IPTG at 37 °C for 15 h. Pelleted cells were resuspended in 300 ml buffer A (30 mM Tris · HCl pH 8.0, 20% sucrose) by gentle shaking and ethylenediamine tetraacetate was added dropwise to a final concentration of 1 mM. After 15 min cells were pelleted by centrifugation, resuspended in 200 ml 5 mM $MgCl_2$, stirred for

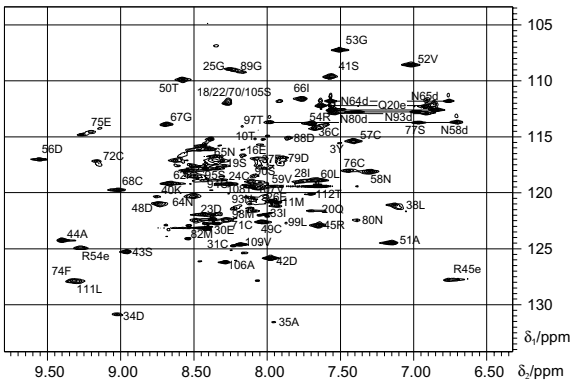

Figure 1. ^{1}H-^{15}N HSQC spectrum (600 MHz) of 0.8 mM His_6PSCD4 of Pleuralin-1 in a mixed solvent of 90% H_2O and 10% D_2O with assignments (pH 6.5, 10 mM sodium phosphate buffer, 298 K).

30 min and pelleted again. The resulting supernatant is the periplasma extract. The extract was supplemented with 5 mM imidazole, 1 ml of Ni-NTA resin (Qiagen) was added and the suspension was incubated for 2 h at 4 °C with constant gentle shaking. The suspension was poured into a column and the resin was washed with 10 ml of buffer B (1 M NaCl, 50 mM Tris·HCl pH 8.0, 20 mM imidazole). Elution of pure His_6PSCD4 was achieved by increasing the imidazole concentration in buffer B to 200 mM. The eluate was dialysed against three changes of 10 mM sodium phosphate pH 6.5, and concentrated to 10 mg/ml by ultrafiltration on a Microsep 3K membrane (Pall Filtron).

The NMR data of His_6PSCD4 were recorded on Bruker DRX-600 and DRX-800 spectrometers at 298 K. Chemical shifts were referenced to internal DSS for ^{1}H resonances. In the case of ^{13}C and ^{15}N resonances, indirect calibration according to the IUPAC recommendations (Markley et al., 1998) was performed. Sequence specific resonance assignment was obtained on the basis of HNCA, CBCA(CO)NH, ^{1}H-^{15}N-HSQC, ^{1}H-^{15}N-NOESY HSQC, ^{1}H-^{15}N-TOCSY HSQC, HNCO, and HACACO spectra. Due to the large number of proline residues of His_6PSCD4, the proline-edited CDCA(NCO)CAHA spectrum (Bottomley et al., 1999) was also recorded. A HCCH-TOCSY spectrum was used to assign side chain resonances.

Extent of assignments and data deposition

The chemical shifts of 97% of the backbone $^{1}H^{N}$, $^{15}N^{H}$ (without consideration of the proline residues), $^{13}C^{\alpha}$, 75% of the $^{13}C'$, 94% of the $^{1}H^{\alpha}$, and 51% of all side-chain atoms of the PSCD4 domain were assigned (data were deposited in the BioMagResBank, http://www.bmrb.wisc.edu/, under accession number 4958). A 2D ^{1}H-^{15}N-HSQC spectrum of His_6PSCD4 recorded on the DRX-600 spectrometer is shown in Figure 1. The distribution of $^{1}H^{N}$ and ^{15}N chemical shifts observed for the backbone amide groups indicates that the protein exhibits a defined structure. This is to be expected, since the structure of the His_6PSCD4 domain is stabilised by five disulphide bridges. From the chemical shift index (CSI) calculated using the computer program csi v. 1.1 (Wishart and Sykes, 1994; courtesy of Sykes et al., http://www.pence.ualberta.ca/ftp), canonical secondary structure elements could not be identified. It should be noted that the chemical shifts of amino acid residues preceding proline residues have been corrected as suggested by Wishart et al. (1994). Nevertheless, the CSI method may fail in this proline-rich protein.

Acknowledgements

We thank R. Glockshuber (ETH Zürich) for providing expression vector pRbi-DsbC and the Deutsche Forschungsgemeinschaft for financial support (SFB 521 and a Heisenberg fellowship to E.B.).

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