Journal of Biomolecular NMR, **14:** 195–196, 1999.
KLUWER/ESCOM

Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the *a′* domain of protein disulfide isomerase

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Received 1 February 1999; Accepted 13 April 1999

Key words: disulfide bond, prolyl-4-hydroxylase, protein disulfide isomerase, protein folding, thioredoxin

Biological context

Protein disulfide isomerase (PDI) is a 55 kDa multifunctional protein mainly involved in the catalysis of disulfide bond formation, breakage and rearrangement. It also functions as a chaperone and a subunit in the enzymes prolyl-4-hydroxylase and the microsomal triglyceride transfer protein (see Kivirikko and Pihlajaniemi, 1998). The amino acid sequence of PDI indicated the presence of four structural domains (Edman et al., 1985). Two domains located at the N- and C-terminus of the PDI sequence, designated ***a*** and ***a′***, were clearly homologous to each other and to the ubiquitous redox protein thioredoxin. NMR structural studies of PDI ***a*** indeed confirmed the expected thioredoxin-like structure (Kemmink et al., 1996). The middle section of the PDI sequence comprises two other domains, termed ***b*** and ***b′***, which are homologous to each other (Edman et al., 1985). Although there is no significant sequence similarity between these domains and either the ***a*** or ***a′*** domain, structural studies using NMR spectroscopy revealed that the ***b*** domain of PDI also adopts a thioredoxin fold (Kemmink et al., 1997).

Both the individual ***a*** and ***a′*** domains are good catalysts of disulfide bond formation. However, efficient catalysis of rearrangement of disulfide bonds requires the presence of other domains. The ***b′*** domain of PDI appears to play an essential role in efficient rearrangement, but other domains are required depending on the complexity of the peptide substrate (Darby et al., 1998). In particular the ***a′*** domain plays an intriguing role different from the ***a*** domain. Furthermore, the ***a′*** domain has been shown to be critical for the assembly of the prolyl-4-hydroxylase tetramer (Koivunen et al., 1999). This prompted us to initiate NMR structural studies of the ***a′*** domain of PDI.

Methods and results

The clone used for the expression of the PDI ***a′*** domain included residues D348-G462 of PDI cloned in pET12a (Novagen). The protein was expressed in *E. coli* strain BL21(DE3) pLysS (Novagen) and purified as described by Darby and Creighton (1995) with some modifications. For preparation of the ^{15}N-labeled sample, 5.0 L of minimal medium containing 1 g/L $^{15}NH_4Cl$ (Isotec) and supplemented with 1xBME Vitamin Solution (Gibco), 1 mM $CaCl_2$, 2 mM $MgSO_4$ plus 50 μg/mL carbenicillin and 34 μg/mL chloramphenicol was inoculated from an overnight culture. Cells were grown until the absorbance at 600 nm reached 0.4–0.5 and the gene expression was induced by addition of IPTG to a concentration of 0.5 mM. The cells were harvested after 4–5 h by centrifugation and frozen. The cell pellets were suspended in 20 mL of 50 mM Tris (pH 8), 1 mM EDTA, 10 μM leupeptin and 0.1 mM PMSF and sonicated. The sonicated cells were centrifuged

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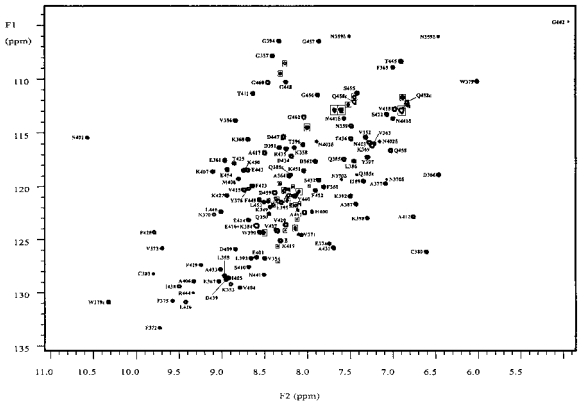

Figure 1. The ^{1}H-^{15}N HSQC spectrum of $^{13}C/^{15}N$-enriched PDI ***a′*** domain in the dithiol form recorded at 300 K. The backbone NH correlations are indicated according to the type of amino acid and the sequence numbering of intact PDI. The extra peaks are enclosed in boxes.

at 90000g for 60 min and DTT was added to a final concentration of 20 mM. The sample was applied onto a 15 × 1.5 cm Q-Sepharose High Performance column (Pharmacia) equilibrated with 50 mM Tris (pH 8), 2 mM DTT (buffer A) and eluted by a linear gradient to 0.4 M NaCl in the same buffer. Fractions containing most of the ***a′*** polypeptide were applied to a Sephacryl S100 High Resolution gel filtration column (Pharmacia) equilibrated and eluted with buffer A. Finally, the ***a′***-containing fractions were dialyzed against 50 mM Tris (pH 8), 5 mM DTT and concentrated. The $^{13}C/^{15}N$ labeled sample was produced in a similar way starting from a 2.5 L culture supplemented with 4 g/L of D-glucose-$^{13}C_6$ (Isotec).

NMR experiments for resonance assignments of PDI ***a′*** domain were recorded on samples uniformly labeled in either ^{15}N or $^{15}N/^{13}C$ at concentrations of 1–2 mM in 93% H_2O/7% D_2O (v/v) containing 10 mM DTT and 10 mM sodium phosphate buffer (pH 6.8). NMR spectra were recorded on a Varian Unity INOVA 600. Details about the employed NMR experiments mentioned without any further reference can be found in e.g. Cavanagh et al. (1996).

Extent of assignments and data deposition

Sequence specific assignment of the backbone ^{1}H, ^{15}N and ^{13}C frequencies of reduced PDI ***a′*** was accomplished using HNCA, HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH, HBHA(CBCACO)NH and COCAH (Dijkstra et al., 1995) experiments recorded at 300 K. The assignment process was complicated by the presence or appearance during the course of experiments of extra peaks. The temperature studies also solved a number of ambiguities present in the spectra recorded at 300 K. The extra peaks can be classified into two categories: (i) A limited number of peaks disappears after addition of DTT; these peaks are caused by the presence of PDI ***a′*** in the oxidized state and (ii) the other ones most probably originate from irreversible degradation of the sample. More than 98% of all backbone atoms that could possibly be assigned using these experiments were assigned. The backbone $^{1}H^{N}$ and ^{15}N assignments are summarized in the HSQC spectrum shown in Figure 1. Assignment of side chains was checked or extended by correlating the $C^{\alpha/\beta}$ and $H^{\alpha/\beta}$ chemical shifts to the other atoms using the HC(C)H-TOCSY and ^{13}C-NOESY-HSQC experiments. Aromatic side-chain assignments were obtained from (adapted) versions of the ^{13}C CT-HSQC, (HB)CB(CGCD)HD (Yamazaki et al., 1993), (HB)CB(CGCDCE)HE (Yamazaki et al., 1993) and HC(C)H-TOCSY experiments. More than 90% of the side-chain resonances that could possibly be assigned using the experiments performed, have been assigned. The assignments have been deposited with the BioMagResBank, entry number 4302.

References

Cavanagh, J., Fairbrother, W.J., Palmer, A.G. and Skelton, N.J. (1996) *Protein NMR Spectroscopy*, Academic Press, San Diego, CA.

Darby, N.J. and Creighton, T.E. (1995) *Biochemistry*, **34**, 11725–11735.

Darby, N.J., Penka, E. and Vincentelli, R. (1998) *J. Mol. Biol.*, **276**, 239–247.

Dijkstra, K., Kroon, G.J.A., van Nuland, N.A.J. and Scheek, R.M. (1994) *J. Magn. Reson.*, **97**, 213–217.

Edman, J.C., Ellis, L., Blacher, R.W., Roth, R.A. and Rutter, W.J. (1985) *Nature*, **317**, 267–270.

Kemmink, J., Darby, N.J., Dijkstra, K., Nilges, M. and Creighton, T.E. (1996) *Biochemistry*, **35**, 7684–7691.

Kemmink, J., Darby, N.J., Dijkstra, K., Nilges, M. and Creighton, T.E. (1997) *Curr. Biol.*, **7**, 239–245.

Kivirikko, K.I. and Pihlajaniemi, T. (1998) *Adv. Enzymol. Related Areas Mol. Biol.*, **72**, 325–398.

Koivunen, P., Pirneskoski, A., Karvonen, P., Ljung, J., Helaakoski, T., Notbohm, H. and Kivirikko, K.I. (1999) *EMBO J.*, **18**, 65–74.

Yamazaki, T., Forman-Kay, J.D. and Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 11054–11055.