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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of the a' domain of ERp57

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Biological context

Protein disulfide isomerase (PDI) is a multifunctional protein involved in the catalysis of disulfide bond formation, breakage and rearrangement. It also acts as the β-subunit in the enzyme prolyl-4-hydroxylase, an $\alpha_2\beta_2$ tetramer, where it keeps the highly insoluble α subunits of these proteins in a catalytically active, nonaggregated state (Kivirikko and Pihlajaniemi, 1998).

PDI is a modular protein consisting of four main domains *a*, *b*, *b′* and *a′*, and a C-terminal extension *c*. Domains *a* and *a′* are similar in their amino acid sequence to each other and to the redox protein thioredoxin.

ERp57 is a PDI isoform that functions as a chaperone in the glycoprotein synthesis. It likewise is a modular protein with four domains, two of which are thioredoxin-like (*a* and *a′*). Despite a high degree of similarity, it cannot substitute PDI as the β-subunit in prolyl-4-hydroxylase (Koivunen et al., 1996). The solution structures of domains *a* and *b* of PDI have been recently determined (Kemmink et al., 1996, 1999), and the sequential assignment of PDI *a′* has been reported (Dijkstra et al., 1999). This prompted us to study the differences of the structures between the domains of PDI and ERp57 and initiate NMR structural studies of the domains of ERp57, in particular the catalytic domain *a′*.

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Methods and experiments

Protein production, purification and preparation of NMR samples. The clone used for the expression of the *a′* domain of human ERp57 included residues S349–E468 of ERp57 cloned in pQE-30 (Qiagen). The polypeptide was expressed in *E. coli* strain SG13009 [pREP4] and purified under native conditions. The ^{15}N labeled sample was prepared by growing the cells in minimal medium containing 1.0 g/l $^{15}NH_4Cl$ (Isotec) as the sole nitrogen source. An overnight culture was inoculated in 2.5 l minimal medium supplemented with 2 mM $MgSO_4$, 100 μM $CaCl_2$, 1× BME vitamin solution (Gibco) and 25 μl/ml kanamycin plus 50 μl/ml carbenicillin. Cells were grown to an optical density of 0.6 at 600 nm, 10 ml of 100× BME vitamin solution was added and protein expression was induced by addition of IPTG to a concentration of 1 mM. The culture was incubated for an additional 4 h and cells were harvested by centrifugation, and frozen. The cell pellets were suspended in 25 ml of ERp1–buffer (20 mM Tris-HCl, pH 7.4; 10 mM imidazole; 20% glycerol), and pulse-sonicated. The sonicated cells were centrifuged at 10 000 $\times g$ for 30 min, the supernatant was combined with 10 ml of Ni-NTA agarose (Qiagen) equilibrated with ERp1-buffer and mixed by shaking overnight at +4 °C. The protein was purified by standard Ni-affinity chromatographic methods. Fractions containing the *a′* polypeptide were combined, concentrated and applied to a Sephacryl S 100 gel filtration column (Pharmacia Biotech). Finally the *a′*-containing fractions were dialyzed against 10 mM NaH_2PO_4 (pH 7.0) and concentrated. The

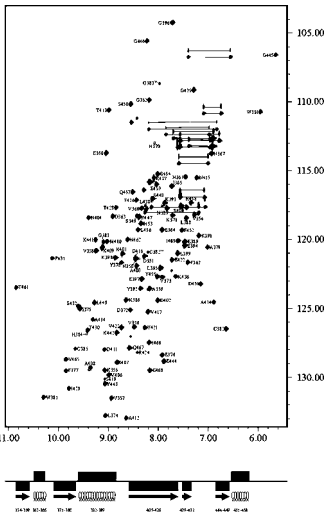

Figure 1. ^{1}H-^{15}N-HSQC spectrum (800 MHz) of a' domain of ERp57 with backbone assignments indicated. Correlations of side-chain amide groups are shown by horizontal lines but have not been assigned. The lower panel shows the prediction of secondary structures by CSI.

^{15}N/^{13}C labeled sample was produced in a similar way using 4 g/l of D-glucose-$^{13}C_6$ (Isotec) as the sole carbon source. The NMR samples of domain a' were prepared at concentrations of 1.4–2.6 mM in 91% H_2O/9% D_2O containing 10 mM dithiotreitol (DTT), and sodium phosphate buffer (pH 6.16).

NMR measurements. ^{1}H-^{15}N-HSQC, HNCACB, CB CA(CO)NH, HNCO, HCCH-TOCSY, CC(CO)NH, ^{15}N-edited TOCSY and NOESY, and ^{13}C-edited NOESY (Grzesiek and Bax, 1992; Yamazaki et al., 1994) were acquired with Varian Inova 600 and 800 MHz spectrometers at 30 °C. Data were processed with cos^2 functions in all dimensions and with linear prediction in the ^{15}N and ^{13}C domains in the triple resonance experiments. All spectra were processed on Silicon Graphics O2 workstations using either Felix97 (FELIX 97.0, Biosym/MSI, San Diego, 1997), or VNMR6.1 software. The XEASY program (Bartels et al., 1995) was used for spectral assignment.

Extent of assignments and data deposition

The 800 MHz ^{1}H-^{15}N-HSQC spectrum of ERp57 a' domain is shown in Figure 1 (top). A nearly complete backbone assignment was obtained for ERp57 a' using triple resonance methods (Grzesiek and Bax, 1992; Kay et al., 1994; Muhandiram and Kay, 1994). Thus, amide ^{15}N frequencies were obtained from a high-resolution 800 MHz ^{1}H-^{15}N-HSQC spectrum, followed by the collection of corresponding intra- and interresidual Cα/Cβ connectivities from 800 MHz HNCACB and CBCA(CO)NH spectra. The carbonyl shifts were obtained from HNCO. Side-chain proton and carbon chemical shifts were assigned using a combination of three spectra. Partial proton and carbon side-chain assignments obtained from [^{15}N]-edited TOCSY, HCC(CO)NH, and CC(CO)NH were subsequently complemented with those from HCCH-COSY and -TOCSY. Chemical shifts of all assigned ^{1}H, ^{13}C and ^{15}N nuclei have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu), entry BMRB 4908.

Hα, Cα and C′ chemical shift indices (Wishart et al., 1992) indicate that ERp57 a' is composed of five β-strands and three α-helices; the consensus CSI predicts β-strands for residues 354–359, 371–380, 405–426, 429–432, 444–449 and α-helices for residues 362–366, 382–400, 451–458 (Figure 1, bottom). Notably, the CSI of PDI a' predicts very similar secondary structural elements, except that PDI a' has a fourth α-helix between the third and fourth β-strands. This α-helix seems to be missing from domain a' of ERp57.

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