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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of poplar phloem glutaredoxin

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Biological context

Glutaredoxins (Grxs) are thiol disulfide oxidoreductases of the thioredoxin super-family. Their function is necessary for the maintaining of the thiol redox status of the cells. Grxs are ubiquitous proteins present in all organisms from bacteria to humans. Through a CP(Y/F)C active site, Grxs catalyse the reduction of disulfide or glutathione (GSH) mixed disulfide with GSH, NADPH and disulfide reductase, by a dithiol or a monothiol based mechanism, respectively (Holmgren et al., 1995). The various functions and targets of Grxs are rather well characterised in bacteria, yeast and mammals (Fernandes and Holmgren, 2004). On the other hand, the plant Grxs are much less known.

Recently, a poplar phloem Grx has been cloned and overproduced (Rouhier et al., 2002a). The 112 residue protein is active in the HED (2-hydroxydisulfide) and dehydroascorbate reduction assays (Rouhier et al., 2002b). The most interesting feature of poplar Grx is its capacity to be a good electron donor for a poplar phloem peroxiredoxin (Rouhier et al., 2001). Peroxiredoxins (Prxs) are novel peroxidases that reduce hydroperoxides without redox cofactors, using a strictly conserved catalytic thiol (Wood et al., 2003). Oxidised Prxs are regenerated by various reducing systems, such as the thioredoxin (Trx) system. The poplar Prx was the first Prx shown to be reduced *in vitro* by the Grx system. Futhermore, this cytosolic Prx can also accept electrons from a poplar Trx (Rouhier et al., 2001). To better understand the molecular interactions in the particular poplar Prx-Grx system, we have assigned the backbone ^{1}H, ^{15}N, ^{13}C resonances and most of the ^{1}H side chain resonances of the poplar Grx. The resonance assignment of the dimeric poplar Prx is presented in the following Letter to ditor. The ^{1}H,^{13}C and ^{15}N resonances assignment of the two proteins is the preliminary work of the Grx-Prx interaction study.

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Methods and experiments

The cDNA encoding poplar Grx was cloned into the pET-3d plasmid vector. *Escherichia coli* BL21 (DE3) cells were transformed with the recombinant plasmid pET-Grx. One ampicillin resistant colony was grown at 37 °C in LB medium supplemented with ampicillin and protein expression was induced for 4 h with 100 μM IPTG when the exponential phase was reached. The protein was expressed and purified as previously described by Rouhier et al. (2002a). Simply labeled [^{15}N] Grx was produced in M9 medium with $^{15}NH_4Cl$ (1 g/l). Doubly labeled [^{13}C,^{15}N] Grx was expressed in the same medium with [$^{13}C_6$]-D-glucose (4 g/l). All NMR samples contained about 0.9 mM poplar Grx in 50 mM phosphate buffer (pH 7.2), 90% H_2O/10% D_2O, 0.02% NaN_3 and 1 mM DTT.

All spectra were recorded at 28 °C on a Bruker Avance DRX-500 and a Varian Innova 800 spectrometers equipped with triple resonance ^{1}H/^{13}C/^{15}N probes. NMR data were processed using NMRPipe (Delaglio et al., 1995) and analysed with

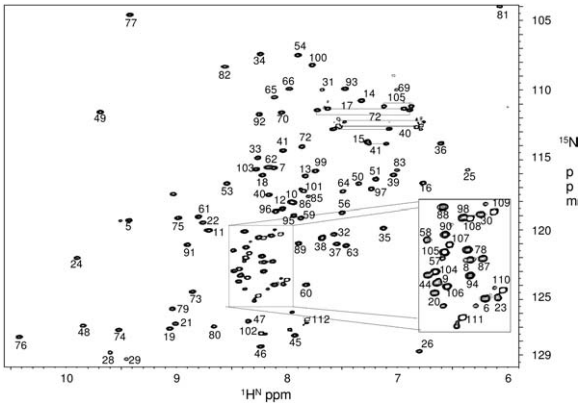

Figure 1. ^{1}H-^{15}N HSQC spectrum of $U-^{15}$N Grx at ^{1}H = 800 MHz, pH 7.2 and 28 °C. Assignments are indicated by residue sequencial number.

PIPP (Garrett et al., 1991) and NMRView (Johnson et al., 1994). ^{1}H dimensions were referenced to DSS and ^{13}C, ^{15}N dimensions were referenced indirectly. Sequence-specific backbone resonance assignments were achieved using the following experiments : HNCA, HN(CO)CA, HNCO, HNCACB and CBCA(CO)NH. Additional chemical shift data were obtained from 2D COSY, TOCSY and NOESY spectra (aromatic and aliphatic proton) and from HNHA experiment. The sequential backbone assignment and ^{1}H chemical shifts were confirmed using ^{1}H-^{15}N NOESY-HSQC spectrum. The R_1, R_2 relaxation rate and ^{1}H-^{15}N NOE measurements were performed at 28 °C (Farrow et al., 1994). All NMR experiments but the ^{1}H-^{15}N NOESY-HSQC and the HNHA experiments were recorded on the Bruker DRX-500 spectrometer.

Extent of the assignments and data deposition

The ^{15}N-HSQC spectrum for reduced poplar Grx is shown in Figure 1. Backbone amide resonances were assigned for all non-proline residues except Q17, H55, S83 and D84 (96% completed). The assignment reaches 89% for ^{1}Hα, 98% for ^{13}Cα, 88% for ^{1}Hβ and 94% for ^{13}CO resonances. The topology of the poplar protein is very similar to the classical Grx fold. Due to the line width of the NMR signals at the concentration used (average R_2 in secondary structure : 15.01 ± 0.48 s^{-1}), the other triple-resonance experiments have a too low sensitivity to assign other ^{13}C resonances. However, most of the side-chain protons could be assigned. Aromatic side chain proton resonances of 10 aromatic residues (out of a total of 12) were assigned. Side chain amide resonances were assigned for all asparagine and glutamine residues except Q50 and Q58. ^{1}H,^{13}C and ^{15}N chemical shifts for poplar Grx have been deposited in the BioMagResBank under accession number 6118.

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