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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N NMR assignment of the homodimeric poplar phloem type II peroxiredoxin

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Biological context

Peroxiredoxins (Prxs) are a new diverse and ubiquitous family of antioxidant enzymes (Wood et al., 2003). With a moderate catalytic efficiency, Prxs reduce the alkyl hydroperoxides, utilizing the activated thiol group of a strictly conserved cysteine residue which is oxidized into a sulfenic acid, Cys-SOH. The next steps of catalysis vary with the Prx sub-type. In the 2-Cys Prxs, the Cys-SOH reacts with another cysteine to form a disulfide bridge, which is in turn reduced by an electron donor such as thioredoxin, tryparedoxin or Ahp D (Hofmann et al., 2003). The 1-Cys Prx are directly reduced by a reacting thiol of a substrate. In general, the electron donor substrate specificity is far from being understood.

Recently, the first Prx shown to be reduced in vitro both by a thioredoxin (Trx) and a glutaredoxin (Grx) systems was characterised. This new Prx is a poplar phloem Prx of type II with a unique cysteine (Cys51) participating to peroxidase activity (Rouhier et al., 2002). Interestingly, other Prxs found in plant (Brehelin et al., 2003) or in Prx-Grx hybrides of some pathogenic bacteria (Rouhier and Jacquot, 2003) were recently shown to be reduced by Grxs. According to the peptide sequence, the poplar Prx is similar to the human Prx V and the yeast Prx Ahp1, both belonging to the D-type Prxs (Trivelli et al., 2003). Nevertheless, neither of these Prxs were regenerated by Grx system. The assignment of the poplar Prx presented here is as the first step of the study of the Prx-Grx and Prx-Trx interaction of the poplar system using NMR. The X-ray structure of the poplar Prx has been resolved and will be published elsewhere (Echalier et al., 2002).

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Methods and results

The expression and purification of the protein was done as previously described (Rouhier et al., 2002). Peroxiredoxin cDNA was cloned in the expression plasmid pET-3d and transfomed into *Escherichia coli* strain BL21 (DE3). ^{15}N-labeled sample was produced by growing the bacteria in M9 minimal synthetic medium with $^{15}NH_4Cl$ (1 g/L). ^{15}N/^{13}C/50%^{2}H-labeled sample was produced by using $^{15}NH_4Cl$ (1g/L) and [$^{13}C_6$]-D-glucose (4 g/L) and 75:25% (v/v) D_2O:H_2O. All NMR data were acquired at 38 °C with samples containing 1.1 mM ^{15}N-labeled protein or 1.5 mM ^{15}N/^{13}C/50%^{2}H-labeled protein in 50 mM sodium phosphate pH 7.2, 90% H_2O / 10% D_2O, 0.02% NaN_3. The data for assignment were acquired in non reducing media. However, no significant differences were observed for the ^{1}H and ^{15}N chemical shifts and the R_2 relaxation rates in reducing media with a large excess of DTT (10 mM).

NMR experiments were collected at 38 °C on a Bruker *Avance* DRX-500 and Varian *Inova* 600 and 800 spectrometers equipped with triple-resonance ^{1}H, ^{13}C, ^{15}N probes and shielded z-gradients. All ^{1}H dimensions are referenced to the H_2O signal relative to DSS, and ^{13}C and ^{15}N dimensions are referenced indirectly. NMR data were processed and analysed using NMRPipe (Delaglio *et al.*, 1995) and PIPP (Garrett *et al.*, 1991) software packages. H^N, ^{15}N, ^{13}C

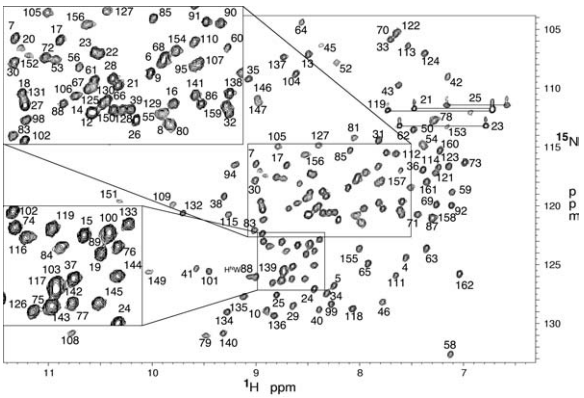

Figure 1. ^{1}H-^{15}N HSQC spectrum of $U-^{15}$N Prx at ^{1}H = 800 MHz, pH 7.2 and 38 °C. Assignments are indicated by residue sequential number.

sequential backbone assignments were achieved using data from the ^{15}N-HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB experiments. ^{1}H and ^{13}C side chain resonances assignment were obtained from (H)C(CCO)NH-TOCSY and H(CCO)NH-TOCSY experiments respectively. ^{1}H-^{1}H TOCSY, ^{1}H-^{15}N NOESY-HSQC and H(CCO)NH-TOCSY were used to assign Hα resonances and confirm ^{1}H side chain assignment. The X-ray structure and long distance NOEs observed could be used for the assignment of ^{1}H aromatic side chains. The R_1, R_2 relaxation rates and the steady-state ^{1}H-^{15}N NOE measurements were performed at 500 MHz and 38 °C with a sample in reducing media (10 mM DTT) (Farrow et al., 1994). For R_1 measurement, spectra were recorded with delays of 22, 55, 155, 255, 500 and 755 ms and spectra duplicated at 155 ms. For R_2 measurement, spectra were recorded with delays of 17, 33, 50, 67 and 83 ms and spectra duplicated at 33 and 67 ms.

Extent of assignments and data deposition

Figure 1 shows the ^{15}N-HSQC spectrum of poplar Prx. The analysis of the ^{15}N relaxation experiments shows that the protein is a stable homodimer, with a rotational diffusion correlation time of 13.1 ns ± 0.1, consistent with a mass of 2 × 17 kDa. All backbone ^{1}H^{N}-^{15}N resonances were assigned, except for Ala2, Phe47, Thr48, Cys51, Lys96, H97, Gly120 and Gly148. Thr48 and Cys51 participate to the catalytic activity and Phe47, Gly120 and Gly148 are near the catalytic site. The assignment of ^{13}CO and ^{13}Cα of Cys51 could be obtained from the HN(CA)CO and HNCA experiments through the correlations from Ser52. The backbone assignment reaches 95% for ^{1}H^{N}, ^{15}N, ^{13}CO, and ^{13}Cα, 93% for ^{13}Cβ, 53% for Hα and 85% of ^{13}C side chain beyond ^{13}Cβ for amino acids with long side chains. The chemical shifts have been deposited in the BioMagResBank under accession number 6132.

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