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# Letter to the Editor: $^{1}H$, $^{15}N$, and $^{13}C$ resonance assignments of reduced glutaredoxin C1 from *Populus tremula x tremuloides*

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## Biological content

Glutaredoxins (Grx) are glutathione-dependent redox enzymes which may operate in essential biosynthetic reactions and regulate many biological functions (Fernandes and Holmgren, 2004). Grxs are ubiquitous proteins which exist in most living organisms from bacteria to humans. In plants, various isoforms of Grx are present, and their biochemical and structural properties are scarce. According to their active site sequences, these Grx isoforms can be separated into three classes: CxxC/S, CGFS, and CCxC/S/G (Rouhier et al., 2004).

Although numbers of Grx structures from *E. coli*., phage T4, pig and human have been determined, no structure of Grx from plants has been reported. Based on the sequence homologies and known Grx structures, the core structure of Grx belongs to a Trx fold. We have started a solution structure determination of a Grx from *Populus tremula x tremuloides* called previously Grx CxxC1 and now shortened to Grx C1. The active site sequence of poplar Grx C1 is YCGYC, which belongs to the CxxC/S class. The sequence identity between Grx C1 and pig Grx (the highest homology in PDB) is about 35%, and Grx C1 displays ca. 10 additional residues at the N-terminus and 5 at the C-terminus. Here we report the complete sequence-specific assignments for the reduced form of poplar Grx C1.

## Methods and experiments

Recombinant poplar Grx C1 was cloned into pET3d vector and transformed into *E. coli* strain BL21(DE3) containing the plasmid pSBET for optimizing the protein expression with rare codons (Rouhier et al., 2002). The bacteria were cultured in $^{13}C/^{15}N$-labeled M9 media at 37 °C, and protein expression was induced for 6 hours with 100 μM IPTG when the exponential phase was reached. The protein was purified by cation exchange chromatography followed by gel filtration. Protein purity was above 90% checked by SDS-PAGE and $^{1}H-^{15}N$ HSQC spectrum. The NMR sample contained about 1.1 mM uniformly $^{13}C/^{15}N$-labeled protein in 40 mM potassium phosphate buffer with 90% $H_2O$/10% $D_2O$ at pH 6.4, along with 40 mM DTT, 0.01% $NaN_3$ and 0.01% DSS, plus Complete, an EDTA-free protease inhibitor cocktail (Roche, Germany). The sample was extensively purged with argon gas to remove oxygen and inhibit oxidation.

NMR measurements were performed at 293 K on a Bruker Avance 500 MHz spectrometer with a triple resonance cryo-probe equipped with a z-axis gradient, and a Bruker Avance 600 MHz spectrometer with a triple resonance probe equipped with a three-axis gradient. Proton chemical shifts

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were referenced to internal DSS. $^{15}N$ and $^{13}C$ chemical shifts were referenced indirectly to DSS using the _ ratios (Markley et al., 1998). The NMR experiments performed include 2D $^{1}H-^{15}N$ HSQC, 3D HNCA, 3D HNCACB, 3D CBCA(CO)NH, 3D HNCO and 3D HN(CA)CO for backbone assignments, 2D $^{1}H-^{13}C$ HSQC, 3D HB-HA(CBCA)(CO)NH, 3D (H)C(CO)NH, 3d H(CCO)NH, 3D $^{15}N$-TOCSY-HSQC, 3d H(C)CH-TOCSY, 3D (H)CCH-TOCSY, 3d H(C)CH-COSY, 3D (H)CCH-COSY for aliphatic side-chain assignments, and 2D (HB)CB(CGCD)HD, 2D (HB)CB(CGCDCE)HE, and 3D $^{1}H-^{13}C$ NOESY-HSQC (optimized for aromatic region) for aromatic side-chain assignments (Sattler et al., 1999). The assignments were further confirmed by 3D $^{1}H-^{15}N$ NOESY-HSQC and 3D $^{1}H-^{13}C$ NOESY-HSQC. The $^{1}H$ TOCSY mixing time is 60 ms, and all $^{13}C$ TOCSY mixing times are 12 ms. All $^{1}H$ NOESY mixing times are 120 ms. The NMR spectra were processed using NMRPipe (Delagio et al., 1995) and analyzed using NMRView (Johnson and Blevins, 1994).

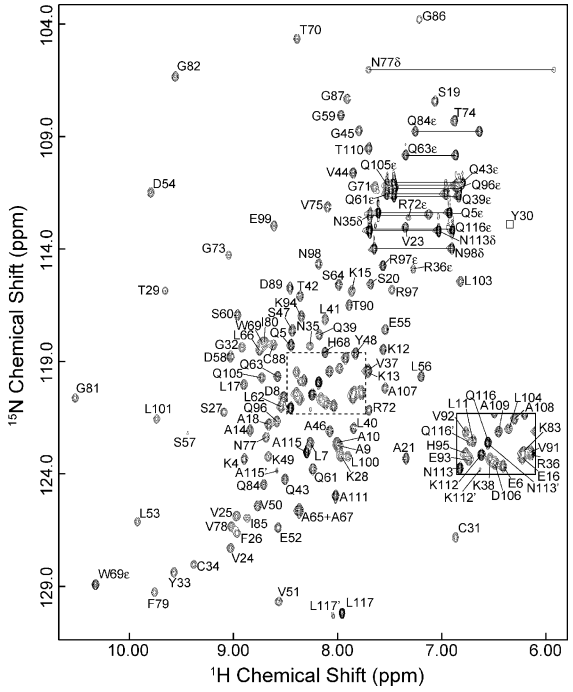


*Figure 1.* 2D $^{1}H^{15}N$ HSQC (600 MHz) spectrum of poplar Grx C1 at 293 K. Assignments are indicated by one-letter amino acid code and the sequence number. Signal from Y30 is not visible at the plot level and the position is indicated by a square. Residues 112–117 show two sets of peaks, which may be due to the *cis-trans* isomerization of the N113–P114 peptide bond.

## Extent of assignments and data deposition

All backbone resonances from non-proline residues were assigned except M1, A2, HN and N of S3 (Figure 1). The aliphatic and aromatic side-chain assignments of non-labile hydrogens are complete except M1 and A2. The labile side-chain protons of Asn, Gln and Arg were assigned by analyzing the HNCA, HNCACB, CBCA(CO)NH and HBHA (CBCA)(CO)NH spectra. Overall, more than 98% of the $^{1}H$, $^{15}N$ and $^{13}C$ resonances were assigned. The $^{1}H$, $^{13}C$ and $^{15}N$ chemical shifts have been deposited in the BioMagResBank under the BMRB accession number 6410.

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