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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignments of the dimeric yeast peroxiredoxin YLR109w

Xavier Trivelli[a], Isabelle Krimm[a,*], Lionel Verdoucq[b], Yvette Chartier[b], Pascale Tsan[a], Yves Meyer[b] & Jean-Marc Lancelin[a,*]

[a]*Laboratoire de RMN Biomoléculaire associé au CNRS, Université Claude Bernard - Lyon1, ESCPE-Lyon, F-69622 Villeurbanne, France;* [b]*Laboratoire de Physiologie Végétale associé au CNRS, Université de Perpignan, F-66860 Perpignan, France*

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Biological context

Peroxiredoxins (Prx) are antioxidant proteins that exert their peroxidase activities through cysteine thiols. Members of this new superfamily have been identified in all organisms (Wood et al., 2003a). They possess a strictly conserved N-terminal catalytical cysteine called peroxidatic which attacks hydroperoxide. An alcohol molecule is then released while the cysteine is oxidised into a sulfenic acid Cys-SOH. The way by which the sulfenic acid Cys-SOH is reduced to complete the catalytic cycle is poorly understood. For the 2-Cys and atypical 2-Cys Prxs, the sulfenic acid is attacked by a second cysteine and an intermolecular or an intramolecular disulfide bridge is formed, respectively. This disulfide bridge is then reduced by thiols such as thioredoxins and glutaredoxins. For the so-called 1-Cys Prxs, the sulfenic acid is stabilised in the 3D structure (Choi et al., 1998). No disulfide bond is formed and the 1-Cys reducer is unknown. Nine crystallographic structures of Prx have been resolved. For the 2-Cys Prxs, the cysteines are brought together to form the intermolecular disulfide bridge by a conformational change that occurs during the catalytic cycle. In the reduced atypical 2-Cys Prx structures, the two cysteines are also too far away to form a disulfide bridge (more than 15 Å), but the structural change is unknown.

Recent studies have pointed out the importance of the peroxiredoxin oligomeric state (Wood et al., 2002). A relation has clearly been found between the oxidation state of the peroxidatic cysteine of the 2-Cys Prxs and the preferred oligomeric state of the protein. Although some recent studies have provided important insights into the field, it is clear that the mechanism and regulation of peroxiredoxins are far from being understood (Wood et al., 2003b). As the first NMR study of a member of this family, we report here the backbone resonance assignment of a dimeric yeast peroxiredoxin, YLR109w. This Prx is particular as the second cysteine involved in the hydroperoxide reduction mechanism has no equivalent in the other known Prxs (Verdoucq et al., 1999). This assignment will therefore be the basis of interaction and dynamic studies.

*To whom correspondence should be addressed. E-mail: lancelin@hikari.cpe.fr

Methods and experiments

YLR109w of *Saccharomyces cerevisiae* was expressed in *E. coli* strain BL21 (DE3) transformed with plasmid pET16b (Novagen). The bacteria were grown in 10 ml of unlabelled LB medium, then transferred into 150 ml and later into 1.5 l of labelled M9 medium. Labelled M9 medium contained 1 g l^{-1} 99% $^{15}NH_4Cl$, 4 g l^{-1} 99% [$^{13}C_6$]-D-glucose, 75% (v/v) 99% D_2O. The protein was expressed and purified as previously described (Verdoucq et al., 1999) and concentrated in 50 mM potassium phosphate, 90% H_2O /10% D_2O, 0.02% NaN_3, 5 mM DTT, pH 5.8, by ultrafiltration on a Microcon column (Amicon-Millipore) to 0.5 ml and $\sim$ 1 mM of uniformly ^{15}N, ^{15}N/^{13}C, or ^{15}N/^{13}C/50% ^{2}H labelled YLR109w.

NMR experiments were collected at 38 °C on Bruker *Avance* DRX-500 and Varian *Inova* Unity+

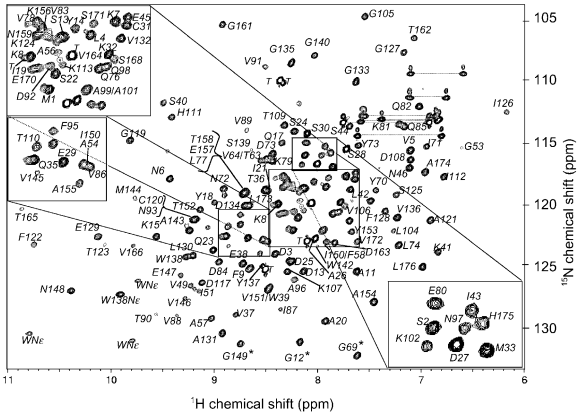

Figure 1. ^{1}H-^{15}N HSQC spectrum of YLR109w at ^{1}H=800 MHz. A *T* letter indicates resonances from the N-terminal His-tag. Folded resonances of three folded Gly are asterisked and Trp HNε1 signals are marked 'WNε'.

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. Sequential backbone assignments were achieved using data from the following experiments: ^{15}N-HSQC, HNCO, d-HN(CA)CO, d-HNCA, d-MQ-HNCOCA, d-HN(CA)CB, d-HN(COCA)CB, d-(H)C(CCO)NH-TOCSY. The assignments were confirmed through sequential NOEs from the 3D ^{1}H-^{15}N NOESY-HSQC spectrum collected with a mixing time of 120 ms. NMR spectra were processed and analyzed using NMRPipe (Delaglio et al., 1995) and PIPP (Garrett et al., 1991) software packages. The R_1, R_2 relaxation rates and the steady-state ^{1}H-^{15}N NOE measurements were performed at 500 MHz at 38 °C (Farrow et al., 1994). For R_1 measurement, spectra were recorded with six inversion recovery delays of 22, 55, 155, 255, 500 and 755 ms and spectra duplicated at 155 ms. For R_2 measurement, spectra were recorded at five Carr-Purcell-Meiboom-Gill delays of 17, 33, 50, 67 and 83 ms and spectra duplicated at 33 and 67 ms.

Extent of assignments and data deposition

The backbone assignment was performed for residues 1 to 176 of the peroxiredoxin YLR109w from *Saccharomyces cerevisiae*. Figure 1 shows the ^{15}N-HSQC spectrum of the protein. All expected backbone $^{1}H^{N}$-^{15}N connectivities could be observed and assigned, except for Ile50, Ser59, Cys62, Ser65, His66, Ile67, Val89, Thr90, Trp100, Phe114, Ala115, Ser167 and Val 169. The backbone assignment reaches 92% for ^{15}N and $^{1}H^{N}$, 95% for ^{13}Cα and ^{13}CO, and 90% for ^{13}Cβ. All ^{13}Cα and ^{13}Cβ of both cysteines involved in the mechanism have been assigned. High values for R_2 and low values for R_1 were calculated from ^{15}N relaxation experiment analysis (19.7 ± 1.9 and 0.81 ± 0.08 Hz, respectively, in secondary structural elements) and a τc of 18 ns was determined, in agreement with a dimeric state of the protein in its reduced form as confirmed by analytical ultracentrifugation. The backbone shifts have been deposited in the BioMagResBank under accession number 5816.

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