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Letter to the Editor: Resonance assignments and secondary structure of hPrxVI, a 25 kDa 1-cys human peroxiredoxin enzyme

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Biological context

Peroxiredoxins (Prxs), which are a family of thiol-specific antioxidant proteins, are also known as thioredoxin peroxidases (TPx) or alkyl-hydroperoxide reductases (Wood et al., 20031). Prxs are divided into three classes, the 1-Cys, atypical 2-Cys, and 2-Cys Prxs, based on the number and position of cysteine residues directly involved in enzyme catalysis. 2-Cys Prxs contain a well conserved cysteine residue in the N-terminal region, which is a peroxidatic cysteine. Human Prx VI (hPrxVI) belongs to the distinct class of 1-Cys Prxs, which contains only an N-terminal conserved cysteine residue, and cannot use thioredoxin. Even though a physiological reducer for hPrxVI is still unknown, it has been shown that hPrxVI mediates the reduction of hydrogen peroxide with the use of electrons from a nonphysiological electron donor, dithiothreitol (DTT) (Kang et al., 1998). X-ray crystal structure of the C91S mutant form of hPrxVI has been determined as a dimeric conformation, where the interface between monomers is apparently composed mainly by hydrophobic interactions and a hydrogen bonding network (Choi et al., 1998). Although a number of reports have suggested the importance of a structural understanding of 1-Cys Prx enzyme, no structural study has yet been performed for native hPrxVI enzyme. Here, we report the resonance assignments and secondary structure of the monomeric hPrxVI, a 25 kDa human peroxiredoxin VI (1-Cys Prx) in the reduced state.

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Methods and experiments

Human PrxVI in the reduced form ($hPrxVI^r$) was expressed in *E. coli* strain BL21(DE3) transformed with plasmid pET17B. Uniformly [$^2H/^{13}C/^{15}N$]-, [$^2H/^{15}N$]- and [30%$^2H/^{13}C/^{15}N$] labeled protein samples were prepared by growing cells in D_2O M9 minimal media containing $^{15}NH_4Cl$, either with or without $^{13}C_6$-D-glucose as the sole source of nitrogen and carbon. Uniformly [$^{13}C/^{15}N$]-labeled protein samples were also prepared by growing cells in M9 media containing $^{15}NH_4$ Cl and $^{13}C_6$-D-glucose. The protein was purified as previously described (Kang et al., 1998). The purified protein was concentrated to 1.5 mM in 50 mM potassium phosphate, 1 mM DTT solution at pH 7.4 with Centricon-10 concentrators (Millipore). All NMR experiments were performed at 30 °C on Bruker DRX600 and DRX800 spectrometers equipped with a triple resonance probe head with gradients. All spectra for backbone resonance assignment were collected using deuterium-decoupled TROSY-based triple-resonance pulse sequences (Pervushin et al., 1997) and processed using NMRPipe/NMRDraw software (Delaglio et al., 1995). Processed spectra were further analyzed with the program Sparky. The assignment was started by using two-dimensional (2D) $^1H-^{15}N$ HSQC and 3D HNCO spectra, to obtain HN(i) and $^{15}N(i)$ resonance frequencies and to resolve

resonance overlap. The backbone assignments were obtained using the TROSY-based HNCA, HN(CO) CA, HNCACB and HN(CO)CACB spectra. Backbone carbonyl assignments were derived from the HNCO and HN(CA)CO spectra. The resonance assignment was confirmed by sequential NOE data from the ^{15}N-edited 3D NOESY-HSQC spectrum. Proton chemical shifts were referenced directly to internal DSS, while ^{15}N and ^{13}C shifts were indirectly referenced (Markley et al., 1998).

Extent of assignments and data deposition

The program AUTOASSIGN (Zimmerman et al., 1997) was applied to obtain initial assignments and further manual methods were used to complete the backbone assignment of all remaining resides. Most of expected backbone NH–^{15}N resonances were unambiguously assigned, except for five residues (Asn13, Phe43, Ser83, Glu159 and Lys216). About 98% of C$^{\alpha}$, ^{13}CO, NH and ^{15}N, and C$^{\beta}$ resonances were assigned and side-chain resonance assignments were completed using data from HCCH-TOCSY and ^{15}N-edited HSQC-TOCSY spectra. About 94% of the side-chain proton resonance assignment was complete except those of aromatic residues. Figure 1a shows the 2D [^{1}H–^{15}N] TROSY-HSQC spectrum of hPrxVI^{r}. Data from consensus chemical shift indices (Wishart and Sykes, 1994) shows that the secondary structure of hPrxVI^{r} in the reduced state consists of ten β-strands and six α-helices. The secondary structure of the wild-type monomeric hPrxVI^{r} in solution is slightly different from that of the dimeric mutant form determined by X-ray crystallography (Figure 1b). The list of ^{1}H, ^{13}C and ^{15}N chemical shifts has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 6148.

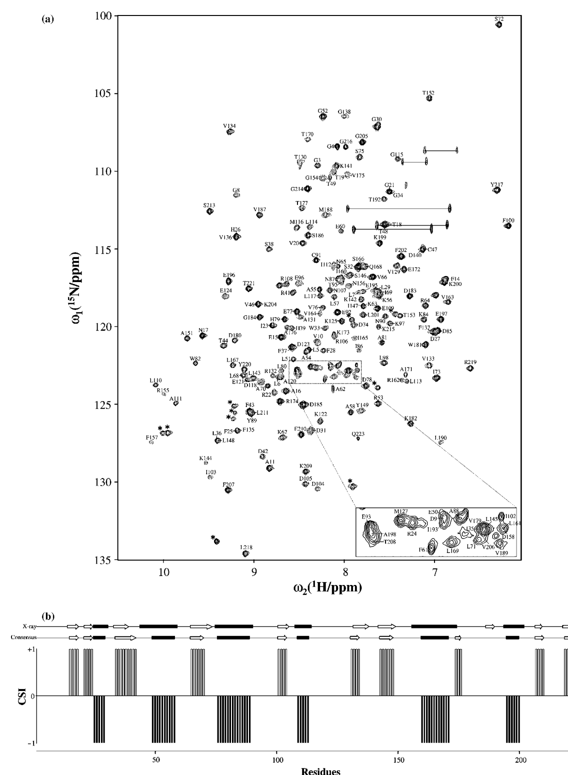

Figure 1. (a) 2D [^{1}H–^{15}N] TROSY-HSQC spectrum of the uniformly [^{2}H/^{13}C/^{15}N]-labeled hPrxVI^{r} (residues 1–224) recorded on a Bruker DRX800 spectrometer at 30 °C, pH 7.4. The cross-peaks connected by lines correspond to side-chain NH_2 groups of Gln and Asn residues. Asterisks indicate peaks for which no sequential or NOE correlations can be identified. (b) Secondary structures of hPrxVI^{r} (NMR) and the C91S mutant form (X-ray). Secondary structure of hPrxVI^{r} was determined by consensus chemical shift indices (CSI). Solid and arrow bars represent α-helical and β-sheet regions of hPrxVI^{r}, respectively.

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