Letter to the Editor: $^1$H, $^{15}$N, and $^{13}$C chemical shift assignments of the human Sulfiredoxin (hSrx)

Duck-Yeon Lee$^a$, Sue Goo Rhee$^a$, James Ferretti$^b$, & James M. Gruschus$^b$*

$^a$Laboratory of Cell Signaling and $^b$Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-0301, U.S.A.

*To whom correspondence should be addressed at: Building 50, Room 3513, South Drive, Bethesda, MD 20892-8013, USA.
Tel.: (301) 496-2350. Fax: (301) 402-3405. E-mail: gruschus@helix.nih.gov

**Key words:** Human sulfiredoxin, heteronuclear NMR assignments, Oxidoreductase
Reactive oxygen species (ROS) are generated along with many cellular metabolisms as by-products. These ROS can oxidize cysteine-thiol in proteins and regulate their function (Finkel, 2000; Rhee et al., 2000). Also, ROS activate signaling pathways related with the oxidative stress response to restore redox homeostasis by increasing the expression of genes encoding antioxidant proteins. Peroxiredoxins (Prxs) are thiol specific antioxidant proteins that catalyze the reduction of H$_2$O$_2$, peroxinitrite and hydroperoxides, which are responsible for oxidation and degradation of lipids, nucleic acids, and proteins (Wood, 2003; Rhee, 2005). During catalysis, the cysteine residue (peroxidatic cysteine) in the active site of Prxs can form a disulfide bond with a neighboring cysteine-thiol (resolving cysteine) or can be hyperoxidized to cysteine-sulfinic acid by H$_2$O$_2$ (Wood et al., 2003). The former is a reversible reaction by cellular antioxidant systems such as glutathione or thioredoxin, but the latter was thought to be an irreversible reaction. Recently, Sulfiredoxin (Srx) has been identified and characterized as the protein responsible for the reduction of cysteine-sulfinic acid to cysteine utilizing ATP in eukaryotes (Biteau et al., 2003). Therefore it seems that cysteine-sulfinic acid formation is also reversible in the cell and might be the sensor system for the oxidative stress by regulating the oxidation status of Prx.

Human sulfiredoxin (hSrx) is a 14kDa and ubiquitous protein but shown varying the expression level among human tissues and can reduce the cysteine sulphinic acid to cysteine in the active site of peroxiredoxin I (PrxI) using not only ATP also GTP (Chang et al., 2004). As the postulated model by Biteau et al., the cysteine-sulfinic acid of Prx is phosphorylated by Srx and ATP in the presence of Mg$^{2+}$ to generate a good leaving group.
that can then be replaced by a thiol. Here we report the backbone assignments of $^{1}H$, $^{15}N$, and $^{13}C$ chemical shift of a 121 residue of hSrx-ΔN16. As Chang, et al. described previously, full-length hSrx is unstable in the solution compare to hSrx-ΔN16 (Chang et al., 2004). Knowledge of the hSrx structure is important to understand the reduction mechanism of Srx.

**Methods and results**

The uniform isotope labeling of $^{13}C$, $^{15}N$ atoms in hSrx was achieved by growing cells in M9 minimal medium with 1.0g of $^{15}NH_{4}Cl$ / L and 2.0g of $[^{13}C]$ glucose / L (Cambridge Isotope Laboratories, Inc. Andover, MA). *E.coli* BL21(DE3) harboring pET15b-hSrx recombinant plasmid was grown at 37°C with vigorous shaking to an OD$_{600}$ of 0.8 and the recombinant protein was induced with 0.5mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 12hr at 20°C. After harvesting cultured cell, cell pellets were resuspended in 50mM Tris-HCl pH 8.0, 300mM NaCl containing 1mM PMSF and lysed by two passes through a French pressure cell. The crude extract was centrifuged at 40000 rpm for 40min and the supernatant was loaded onto a nickel nitrilotriacetic acid-agarose resin (Ni-NTA) column (Qiagen). The polyhistidine tag fused hSrx was eluted with elution buffer (50mM Tris-HCl pH 8.0, and 300mM NaCl) containing 0.3M imidazole. The polyhistidine tag was removed by thrombin for 12hr at room temperature. The cleaved protein solution was dialyzed and loaded again onto a Ni-NTA to remove the polyhistidine and uncleavaged hSrx. After pooled the flow-through fraction, this fraction was concentrated by ultra-filtration using an Amicon ultrafiltration apparatus (Amicon).
The concentrated sample was further purified by gel filtration chromatography using a Superdex G200 column (Pharmacia, 1.6cm x 60cm) in 50mM Tris-HCl pH7.4, 100mM NaCl. We confirmed the molecular mass and purity by mass spectrometry and SDS-polyacrylamide gel electrophoresis (PAGE), respectively. NMR samples were prepared in Shigemi (Shigemi, Tokyo) tubes with 2.5mg of hSrx protein in 250ul of 50mM potassium phosphate pH 7.3, 100mM NaCl and 2mM DTT.

All NMR experiments were performed on a Bruker Avance 800 spectrometer at 300K. The NMR experiments performed included 2D $^{15}$N HSQC, $^{13}$C CT-HSQC ($^{13}$C carrier 40ppm), $^{13}$C HMQC ($^{13}$C 125ppm), 3D $^{15}$N NOESY-HSQC (Gruschus and Ferretti, 1999), $^{13}$C NOESY shortCT(2.7ms)-HSQC ($^{13}$C 40ppm), $^{13}$C NOESY-HMQC ($^{13}$C 125ppm), all NOESY with $t_{mix}$ 100ms, HNCACB and CBCA(CO)NH. All the NMR spectral data were processed and analyzed with nmrPipe/nmrDraw (NIH, Bethesda) (Delaglio et al., 1995).

**Extent of assignments and data deposition**

The full-length hSrx protein showed broad signals in a 1D spectrum and formed considerable precipitate during experiment. Removing the first 16 residues by thrombin prevented precipitation and yielded sharp signals in the 1D spectrum, so this construct was used for NMR. Human srx has one cysteine, and though disulfide formation was not observed, a split cysteine backbone resonance was seen initially; 2mM of DTT was added and only one cysteine resonance was subsequently observed. Figure 1 shows the $^1$H-$^{15}$N HSQC spectrum and assignments of hSrx-$\Delta$N16. Backbone assignments for hSrx-$\Delta$N16 are 100% complete for residues 17 through the C-terminus (residue 137), excluding
carbonyls for which no experiments were performed. The side chain assignments are 98% complete; because of fast exchange due to pH 7.3, only one arginine HN$^\varepsilon$ provided sufficient NOESY signal to assign, and no other arginine, histidine or lysine exchangeable resonances were assigned, though one threonine hydroxyl proton was assigned via cross peaks observed in the $^{15}$N NOESY. A transition from sharp signals with no NOESY cross peaks to broader signals with clear NOESY cross peaks occurs around residue 37, indicating that the N-terminal residues up to residue 37 form flexible, random coil. No such transition occurs at the C-terminus, that is, the C-terminus appears to be a well-ordered part of the structure.
References


Figure 1. Two-dimensional $^1$H-$^{15}$N-HSQC spectrum and assignments of hSrx-$\Delta$N16. Residue numbering is relative to full-length hSrx. All the peaks with $^{15}$N freq less than 105ppm are folded.