



Letter to the Editor: Assignment of ^1H , ^{13}C , and ^{15}N resonances of reduced *Escherichia coli* glutaredoxin 2

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Biological context

The thioredoxin and glutaredoxin families participate as hydrogen donors in a wide variety of cellular reactions (Holmgren, 1989). Glutaredoxins are distinguished from thioredoxins by their ability to catalyze glutathione (GSH)-disulfide oxidoreductions via two redox-active cysteine residues (Holmgren and Åslund, 1995). Glutaredoxin 2 (Grx2) is one of the three glutaredoxins found in *E. coli*, but unlike the other two, Grx2 is not a hydrogen donor for ribonucleotide reductase (Åslund et al., 1994). It has an unusually large size (24.3 kDa) with 215 amino acid residues, whereas the typical molecular weights for glutaredoxins are around 10 kDa. Grx2 has little sequence homology with other glutaredoxins or thioredoxins, except it contains the conserved active site sequence of Cys-Pro-Tyr-Cys.

Grx2 appears most abundant among all three glutaredoxins in *E. coli*. It may account for about 80% of the glutaredoxin activity in the GSH-disulfide oxidoreductase assay (Vlamis-Gardikas et al., 1997). Although the specific biological function is still unknown, the unique properties of Grx2 indicate that it is likely to have a distinctive function. Grx2 has been overproduced in *E. coli*; the protein was doubly labeled with ^{13}C and ^{15}N , and triply labeled with ^2H , ^{13}C and ^{15}N . Multidimensional NMR spectroscopic studies of the labeled Grx2 have led to extensive backbone and side-chain resonance assignments.

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Methods and results

The DNA fragment encoding Grx2 sequence was cloned into *NdeI/BamHI* sites of the pET16a plasmid vector (Novagen, Madison, WI). The recombinant plasmid was transformed into *E. coli* strain BL21(DE3)/pLysS for protein expression. The bacteria were grown at 35 °C in M9 minimal medium and protein expression was induced with IPTG when the cell density reached $\text{OD}_{600} \sim 0.6$. The bacteria were harvested at about 12 h after induction. This post-induction period is longer than is generally required; this was the result obtained from optimization. The Grx2 was first purified on an anion exchange column, followed by gel filtration. The yield of pure protein was about 100 mg per liter of culture, and very similar yields were obtained in H_2O and D_2O expression media. The protein concentration was determined by UV absorption at 280 nm using an extinction coefficient of $21860 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Vlamis-Gardikas et al., 1997). Doubly labeled [^{13}C , ^{15}N]Grx2 was produced in M9 medium with $^{15}\text{NH}_4\text{Cl}$ (1 g/L) and [$^{13}\text{C}_6$]-D-glucose (4 g/L). Triply labeled [^2H , ^{13}C , ^{15}N]Grx2 was expressed in the same medium prepared in D_2O . The deuteration level for aliphatic hydrogens was about 80% measured with NMR spectroscopy.

All NMR samples contained about 2 mM Grx2 in 50 mM phosphate buffer (pH 7.0), with 20 mM deuterated dithiothreitol (DTT). NMR experiments were recorded at 20 °C on either Bruker AMX500 or DRX600 spectrometers equipped with triple-resonance $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ probes and pulse field gradient capabilities. The Fourier transformation of NMR

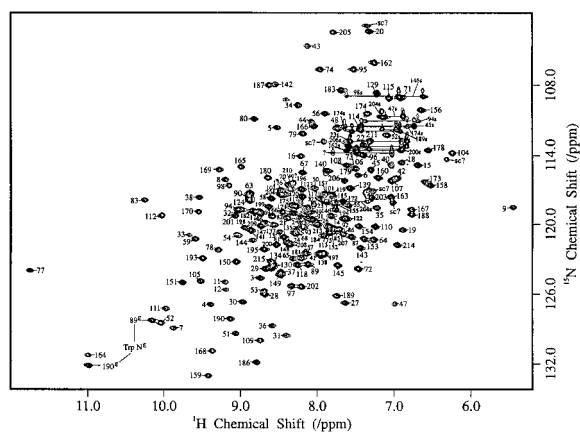


Figure 1. 2D $^1\text{H}\{^{15}\text{N}\}$ HSQC spectrum of a sample of reduced $[U\text{-}^{15}\text{N}, ^{13}\text{C}]\text{Grx2}$ at 20°C. Assignments for cross peaks are indicated by residue number. Cross peaks labeled sc? most probably belong to side chains of lysine or arginine.

data and peak picking were performed with FELIX97 (Molecular Simulations, San Diego, CA). All ^1H dimensions were referenced to internal DSS and ^{13}C , ^{15}N dimensions were indirectly referenced to DSS.

All NMR spectra for the doubly labeled reduced $[^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$ were acquired on a Bruker DRX600 spectrometer, while the spectra for the triply labeled $[^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$ were acquired on a Bruker AMX500 spectrometer. Backbone sequential resonance assignments were obtained using HNCACB and CBCA(CO)NH spectra of the doubly labeled sample. The overall quality of the HNCACB data was not sufficient for more than 50% of the assignments to be made. The sequential assignments were completed using the triply labeled protein; $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ chemical shifts obtained from HNCA/HN(CO)CA and HN(CA)CB/HN(COCA)CB (Yamazaki et al., 1994) experimental data; HNCO/HN(CA)CO spectra were used to confirm the backbone assignments.

The backbone sequential assignment was conducted in a semi-automated manner using the computer program CONTRAST (Olson and Markley, 1994). Assignments for the side-chain resonances of reduced $[^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$ were mainly based on HCCH-TOCSY and HCCH-COSY data with some information from HBHA(CO)NH, H(CCO)NH, and C(CO)NH data. Figure 1 shows the 2D $\{^1\text{H}, ^{15}\text{N}\}$ HSQC spectrum for reduced Grx2, with assignments for the cross peaks indicated by residue numbers.

There were some systematic differences observed between the ^{13}C chemical shifts obtained from proto-

nated and deuterated samples. In general, the $^{13}\text{C}\alpha$ resonances were shifted an average of 0.49 ppm upfield in the deuterated samples relative to the protonated samples. The $^{13}\text{C}\beta$ resonances were shifted upfield by an average of 0.79 ppm.

Analysis of the patterns of NOE connectivities, chemical shift index, and hydrogen exchange data suggests the presence of 4 β -strands and 10 α -helices for reduced Grx2. The structure is mainly helical with four β -strands towards the N-terminus. These form a mixed β -sheet, as indicated by cross strand NOEs (data not shown).

Extent of assignments and data deposition

Backbone sequential assignments have been completed for 95% of the total of 215 amino acid residues using both $[^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$ and $[^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$. The backbone amides for residues 60–62, 126–128, 131–133, 136, 192 and 212 could not be identified, presumably due to the relaxation properties of those regions. About 90% of the aliphatic side-chain ^1H resonances were assigned. The assignments of ^1H , ^{13}C , and ^{15}N resonances in reduced $[^{13}\text{C}, ^{15}\text{N}]\text{Grx2}$ have been deposited in the BioMagResBank database under accession number 4318.

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