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^{1}H, ^{13}C, and ^{15}N NMR resonance assignments of vaccinia glutaredoxin-1 in the fully reduced form

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Biological context

A theme ubiquitous throughout numerous species and particularly within two large DNA viral genomes, vaccinia and T4 phage, is the encoding and packaging of redox-active gluatredoxin(s). Glutaredoxins, initially discovered as an external cofactor to ribonucleotide reductase in *E. coli*, are a class of proteins involved in electron transfer reactions via a pair of active-site thiols. Vaccinia glutaredoxin-1 (vacgrx-1), a product of the *o2l* gene, is a functional thioltransferase and is expressed after the onset of DNA replication (Ahn and Moss, 1992). Very recently, human glutaredoxin has been shown to regulate the activity of HIV-1 protease and to be assembled into HIV viral particles (Davis et al., 1997). Interestingly, vacgrx-1 is also packaged in and associated with infectious vaccinia virions (Ahn and Moss, 1992), suggesting a possible functional role for glutaredoxins in viral pathogenesis. Glutaredoxins show a high degree of homology in the vicinity of the active site, however the homology between *E. coli* glutaredoxin and the vaccinia glutaredoxin breaks down outside these regions. Vaccinia glutaredoxin is longer than its *E. coli* counterpart and contains an additional cysteine residue at the C-terminus. In contrast, mammalian glutaredoxins all contain a pair of additional cysteines at a very different location. Thus, it is of significant interest to structurally characterize this viral form of glutaredoxin. Herein we report the ^{1}H, ^{15}N and ^{13}C sequence specific resonance assignments for vaccinia glutaredoxin-l as a prelude for structural and dynamical investigations of this evolutionarily conserved redox protein.

Methods and results

Vaccinia glutaredoxin was expressed and purified from *E. coli* strain BL21(DE3)pLysS harboring the pET-(GRX) plasmid according to a modification of the procedure described in Ahn and Moss, 1992. A uniformly ^{13}C/^{15}N-labeled 0.8 mM protein sample was prepared by growing the cells in a minimal media containing 15 g/L Na_2HPO_4, 2 g/L KH_2PO_4, 0.5 g/L NaCl, 0.47 g/L Na_2SO_4, 1 mg/L thiamine, 1 mg/L biotin, 0.25 g/L mg_2SO_4, 33 mg/L $CaCl_2$, 100 μg/ml carbenicillin, 20 μg/mL chloramphenicol, 1 g/L $(^{15}NH_4)_2SO_4$ and 3 g/L of a ^{13}C/^{15}N-labeled mixture of sugars and peptides (EMBL, Heidelberg) as the sole source of nitrogen and carbon. The final NMR buffer consisted of 50 mM potassium phosphate, pH 6.5, 0.1 mM EDTA, 5 mM DTT, 0.1% NaN_3 and 5% D_2O.

All NMR measurements were made at 20 °C on a Varian UNITYplus 500 MHz NMR spectrometer equipped with an actively shielded gradient triple resonance probe from Nalorac Corporation and pulsed field gradients. Carrier frequencies were 4.83 ppm for ^{1}H, 116.1 ppm for ^{15}N, 45 ppm for aliphatic ^{13}C, 58 ppm for $^{13}C^{\alpha}$, and 177 ppm for $^{13}C'$.

NMR data were processed using the program PROSA (Güntert et al., 1992). Forward linear prediction was used to extend the time-domain data in the ^{15}N and ^{13}C dimensions. The time-domain data were zero-filled once to enhance the spectral resolution. The chemical shifts were referenced using external DSS (2,2-dimethyl-2-silapentane-5-sulfonate = 0 ppm). Visualization and analysis of spectral data were performed using the program XEASY (Bartels et al., 1995).

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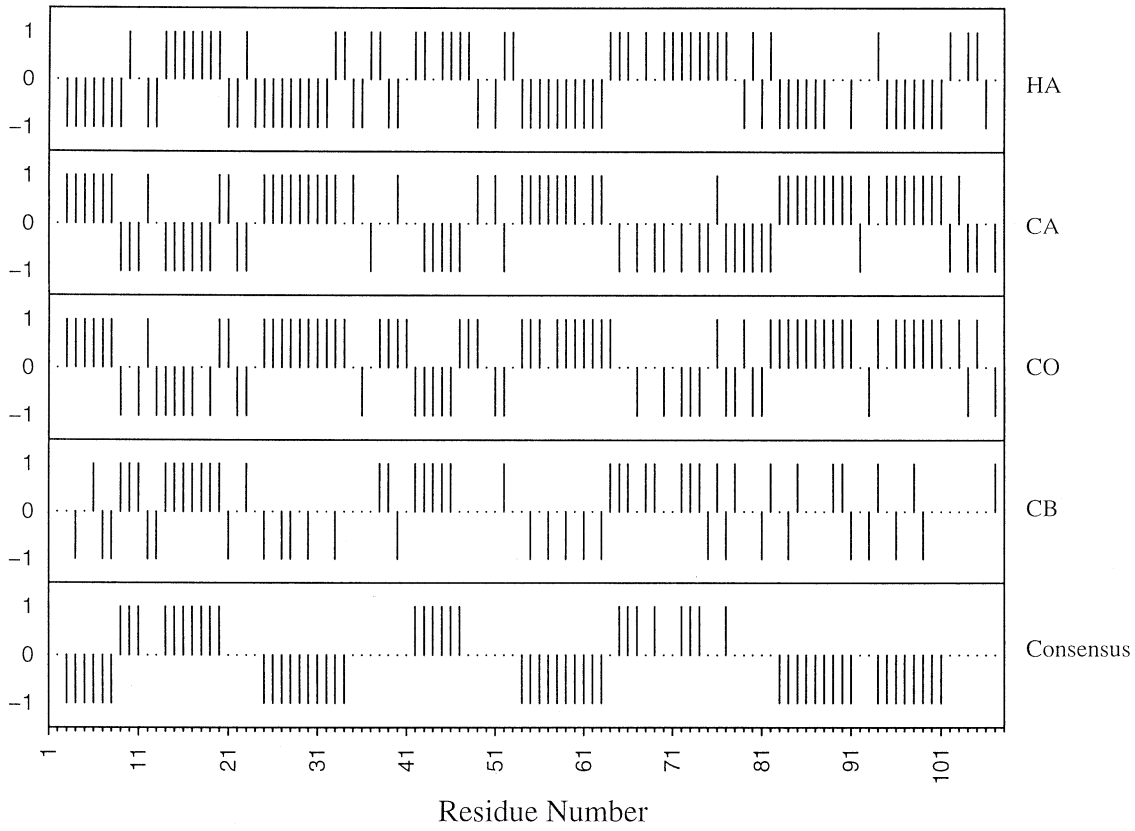

Figure 1. Chemical Shift Index (CSI) data derived from H^{α}, C^{α}, C^{β}, and C' chemical shifts for reduced vaccinia glutaredoxin-1. An index of 0 refers to the random coil structure, an index of −1 represents helical structure, and an index of +1 indicates β-structure for the consensus sequence.

The sequence-specific backbone resonance assignments were based on 3D HNCACB and HN(CO)CACB experiments (Muhandiram and Kay, 1994). H^{α} assignments and additional confirmation of sequential connectivities were obtained from a modified HN(CA)HA (Clubb et al., 1992). Carbonyl carbon ($^{13}C'$) chemical shifts were assigned using data from a 3D HNCO experiment. Assignment of the CH_n moieties in nonaromatic side chains were obtained with 3D HCCH-TOCSY (Kay et al., 1993), 3D C(CO)NH and 3D H(CCO)NH (Grzesiek et al., 1993). Aromatic proton and carbon spin systems were assigned using the 3D HCCH-TOCSY (Kay et al., 1993) experiment and sequence specific connectivities were made from the aromatic ring protons to $C^{\beta}H_2$ using 2D $(H^{\beta})C^{\beta}(C^{\gamma}C^{\delta})H^{\delta}$ and $(H^{\beta})C^{\beta}(C^{\gamma}C^{\delta}C^{\epsilon})H^{\epsilon}$ experiments (Yamazaki et al, 1993).

Wishart and Sykes have developed a chemical shift index (CSI) which quite accurately predicts the secondary structure based on the chemical shifts of the backbone atoms H^{α}, C^{α}, C^{β}, and C'. Figure 1 shows the CSI data obtained for the reduced form of vacgrx-1. There are five α-helical segments spanning residues 3–8, 25–34, 54–63, 83–91, and 94–101. The consensus CSI analysis shows β conformation for residues 9–11, 14–20, 42–47, 65–67, 69, 72–74, and 77. As no C' chemical shift information was obtained for N12, the break between residues 9–11 and 14–20 probably results from the lack of chemical shift information for this region. The CSI of K77 shows an isolated positive deviation characteristic of β conformation. Interestingly, in human glutaredoxin there is a short β-strand in this region which the consensus CSI failed to identify. The overall pattern of secondary structure closely resembles that seen in human glutaredoxin (Sun et al., 1997), thus the viral glutaredoxins appear to mimic the mammalian proteins in terms of their structures. We are currently pursuing the determination of the three-dimensional structure of the viral glutaredoxin

to provide a detailed structural comparison to the mammalian systems.

Extent of assignments and data deposition

The ^{1}H, ^{13}C and ^{15}N chemical shifts for vaccinia glutaredoixn at pH = 6.5 and T = 293 K have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4113. Resonances are not assigned for the following residues: M1 (all), A2 (all), N13 ($^{1}H^{N}$, ^{15}N), K14 ($^{13}C^{\gamma}$,$^{1}H^{\gamma}$,$^{13}C^{\delta}$, $^{1}H^{\delta}$,$^{13}C^{\epsilon 1}H^{\epsilon}$), R40 ($^{1}H^{\epsilon}$, $^{15}N^{\epsilon}$), R106 ($^{1}H^{\epsilon}$, $^{15}N^{\epsilon}$).

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