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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignment of the hydroquinone form of flavodoxin from *Desulfovibrio vulgaris* (Hildenborough) and comparison of the chemical shift differences with respect to the oxidized state

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Biological context

D. vulgaris flavodoxin consists of 147 amino acids and has a molecular mass of 16.3 KDa (Dubourdieu and Fox, 1977). The 3D solution structure of oxidized *D. vulgaris* flavodoxin has already been described. The overall fold of the protein consists of five parallel ß strands, surrounded by two pairs of α-helices either side (Knauf et al., 1996). Flavodoxin-like domains occur in larger flavoenzymes such as human NO synthase and cytochrome P450 reductase (Wang et al., 1997). When the FMN is reduced to the semiquinone, a new hydrogen bond is formed. The bond involves N(5)H of FMN and the backbone carbonyl of glycine 61 (Watt et al., 1991), and is thought to contribute to the greater stability of the semiquinone. The carbonyl group points away from the oxidized FMN in *D. vulgaris* flavodoxin and a protein conformational change occurs when the flavin is reduced. The only changes that are observed upon reduction of flavodoxin is an increase in flexibility of the 3 loop regions; 10–15, 59–62 and 95–102 (Hrovat et al., 1997) which are considered important for flavin binding. A detailed study between the interaction of the apoprotein and the flavin is essential. Here we report the complete assignment of the chemical shifts for the hydroquinone of *D. vulgaris* wild-type flavodoxin and the deposition of the chemical shifts for the oxidized and fully reduced states and compare the chemical shift differences between these two redox states.

Methods and Experiments

Uniformly labelled [^{15}N] and [^{15}N,^{13}C] labelled recombinant flavodoxin from *D. vulgaris* was expressed in *Escherichia coli* TG1 as previously described (Curley et al., 1991) and grown on M9 medium (Knauf et al., 1996). Protein expression was induced by the addition of 20 μM isopropyl-ß-D-thiogalactopyranoside (IPTG) when the cells reached an O.D.$_{600}$ between 0.4–0.5. The protocol for purification is reported elsewhere (Curley et al., 1991). The NMR samples were prepared in 100 mM sodium pyrophosphate pH 8.5 containing 0.02% sodium azide and about 50 μg/ml Pefabloc protease inhibitor as well as 0.15 mM 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). The final protein concentration was approximately 5 mM for the singlely labelled sample and 2.5 mM for the doublely labelled sample.

To obtain the hydroquinone redox-state the protein was made anaerobic by successive cycles of degassing and flushing with oxygen free argon. A 3-fold molar excess of sodium dithionite dissolved in the same buffer as the protein was added anaerobically using a gas tight Hamilton syringe.

With the exception of the 2D-NOESY and the 3D-TOCSY-HSQC, which were recorded on a Bruker DMX500 spectrometer, NMR spectra were recorded on a Bruker DMX600 spectrometer using a ^{1}H, ^{13}C, ^{15}N triple resonance probe, which was equipped with a self-shielded x, y and z-gradient coil. All chemical shifts were referenced with respect to the internal standard DSS. All data was recorded at 300 K. The spectra were processed and analyzed on a Silicon Graphics O_2 workstation using XWIN-NMR and AURELIA software packages (Bruker Biospin, Karlsruhe, Germany).

The sequential backbone assignment was made using triple resonance experiments, by linking the *i* and *i*-1 signals in HNCACB and (HCA)CO(CA)NH spectra. Intraresidual ^{13}CO and ^{13}Cα/^{13}Cβ correlations were distinguished from interresidual peaks with the

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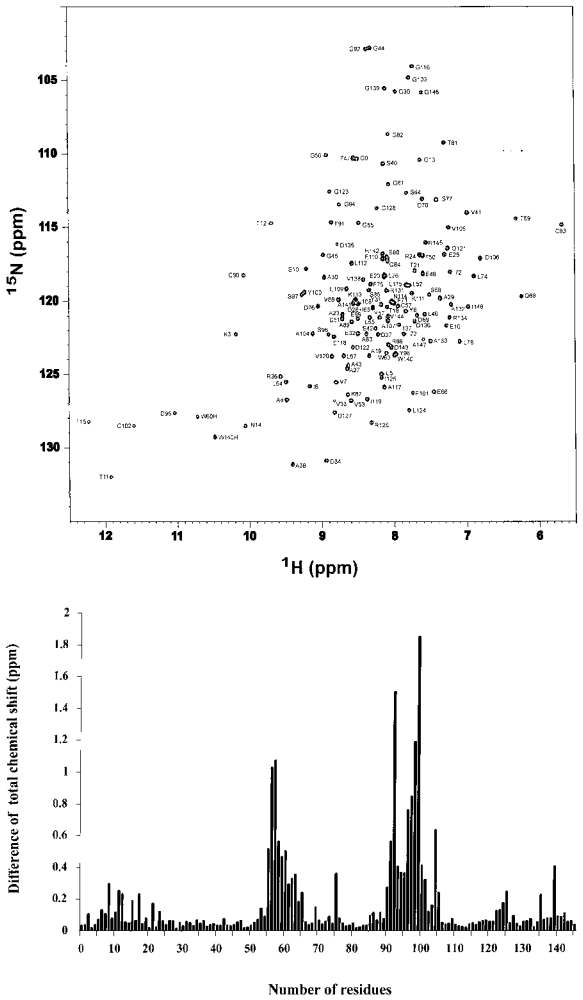

Figure 1. (a) ^{1}H- ^{15}N TROSY spectrum of the 5 mM [^{15}N] labelled flavodoxin in 100 mM sodium pyrophosphate pH 8.5 containing 0.02% sodium azide and about 50 μg/ml Pefabloc protease inhibitor as well as 0.15 mM DSS acquired at 300 K on a Bruker DMX600 spectrometer. Backbone and some sidechain amide cross peaks are indicated with their one letter code and number. (b) Comparison of the oxidized and reduced backbone chemical shifts (HN, N, CO and Cα). The combined chemical shift difference $\Delta\ \delta_{tot}$ on all the residues were calculated according to Equation 1.

help of HNCO and (H)CC(CO)NH-TOCSY experiments, respectively. The latter experiment additionally provided the remaining aliphatic sidechain ^{13}C chemical shifts. H(CC)(CO)NH-TOCSY and TOCSY-HSQC spectra were used to determine sidechain protons. A 2D NOESY was performed to verify serine and cystine proton signals.

Extent of assignment and data deposition

Unambiguous assignment of backbone resonances (HN, N, CO and Cα) was obtained for all residues (except for pro 2, 73 and 130). Sidechain assignment was acomplished for 100% of the aliphatic ^{13}C and over 90% of the ^{1}H resonances.

The ^{1}H, ^{13}C and ^{15}N backbone and sidechain assignments for reduced flavodoxin have been deposited with the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number BMRB-5540, the complete ^{1}H, ^{13}C and ^{15}N backbone and sidechain assignments for oxidized flavodoxin have also been deposited under the accession number BMRB-5571.

Figure 1a shows the [^{15}N,^{1}H] TROSY spectrum of the ^{15}N labelled flavodoxin in the hydroquinone form and Figure 1b shows that significant conformational changes occur between the oxidized and reduced states in the region of residues 58–70 and 94–106, the loops which contain the FMN binding region. These changes were calculated according to equation one.

$$\Delta\delta_{tot} = ((\Delta\delta_{HN}W_{HN})^2 + (\Delta\delta_{N}W_{N})^2 + (\Delta\delta_{CO}W_{CO})^2 + (\Delta\delta_{C\alpha}W_{C\alpha})^2)^{1/2}. \quad (1)$$

The weighting factors used were $W_{HN} = 1$, $W_{N} = 0.154$, $W_{C\alpha} = 0.276$ and $W_{CO} = 0.341$ (Ayed et al., 2001).

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