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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignment of the flavodoxin-like domain of the Escherichia coli sulfite reductase

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Biological context

In *Escherichia coli*, sulfite reductase (SiR) catalyzes the 6-electron reduction of sulfite to sulfide, a key reaction in the sulfate assimilation pathway leading to the biosynthesis of sulfur organic compounds such as L-cysteine (Kredich, 1996). SiR has been originally described as an $\alpha_8\beta_4$ protein where each α-chain (SiR-FP) contains both one FAD and one FMN as prosthetic groups whereas the β-chains (SiR-HP) contain an iron-sulfur cluster coupled to a siroheme. In SiR-FP, the FAD receives electrons from NADPH and the FMN transfers the electrons to the metal centers of SiR-HP where sulfite reduction takes place (Siegel et al., 1974). In SiR-FP, FAD binds to a ferredoxin-$NADP^+$ reductase-like domain and FMN is bound by a flavodoxin-like domain (for a review, see Covès et al., 2000). In order to get more information on the electron transfer mechanism in SiR-FP, we have used a molecular dissection strategy that led to the expression of simplified functional fragments of the native protein (Covès et al., 2000).

Recently, the crystal structure of SiR-FP60, a monomeric but fully active, N-terminally truncated version of SiR-FP (Zeghouf et al., 1998) has been determined (Gruez et al., 2000). Unfortunately, in the four different crystal forms obtained, the flavodoxin-like domain was absent from the electron density. This was interpreted as a flexibility of the FMN-binding domain required for optimal electron transfer. In order to complete the crystal structure, we designed a monomeric version of the flavodoxin-like domain of SiR-FP and now report its ^{1}H, ^{13}C and ^{15}N backbone and sidechain assignment.

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Methods and experiments

The coding sequence, corresponding to residues 52 to 218 of SiR-FP was cloned into the NdeI/BamHI sites of a pET-30c expression vector (Novagen). The resulting protein SiR-FP18 was overexpressed in the *E. coli* strain BL21(DE3). The freshly transformed bacteria were grown at 37 °C in M9 minimal medium containing 1 g/l of $^{15}NH_4Cl$, 2 g/l of ^{13}C-glucose and supplemented with $MnCl_2$ (0.1 mM), $ZnSO_4$ (0.05 mM), $FeCl_3$ (0.05 mM) and a vitamin solution according to Jansson et al. (1996). Protein expression was induced by the addition of 0.5 mM IPTG when cells reached an A_{600} between 0.5 and 0.6. A triple-labelled sample was obtained from growing the bacteria in 90% D_2O. The deuteration level was 77% as deduced from mass spectroscopy. The protein was purified to homogeneity in a single step on a Superdex-75 gel-filtration column (Amersham-Pharmacia) and reconstituted with commercial FMN (Sigma). The final NMR samples were prepared in 100 mM potassium phosphate buffer, pH 7.0 containing 0.02% sodium azide and the protein concentration was about 1.5 mM.

All NMR experiments were performed on Varian INOVA 600 and INOVA 800 spectrometers, both equipped with a triple-resonance (^{1}H, ^{15}N, ^{13}C) probe and shielded z-gradients. The sample temperature was set to 30 °C. All triple-resonance experiments except the two reduced-dimensionality experiments (see below) used the pulse sequences provided by the Varian protein pack (available at ftp site: ftp.nmr.varian.com).

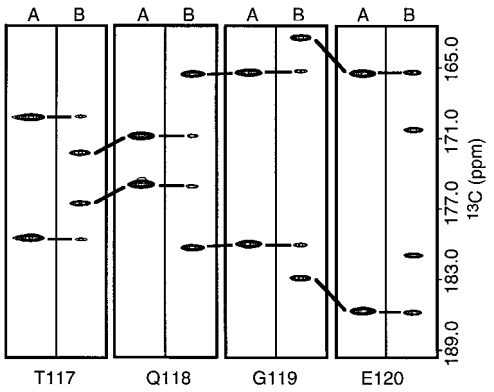

Figure 1. Sequential backbone assignment of SiR-FP18 using reduced-dimensionality triple-resonance experiments: (A) MQ-HNCOCA and (B) MQ-COHNCA. Strips were extracted from the 3D spectra at the $^{1}H^{N}$ and ^{15}N frequencies of the corresponding residue. The CO and C^{α} chemical shifts are obtained from the separation (C^{α}) and the midpoint (CO) of the two peaks in the ^{13}C dimension.

All chemical shifts were referenced with respect to the H_2O signal relative to DSS using the $^{1}H/X$ frequency ratios of the zero point according to Markley et al. (1998).

For the sequential backbone assignment, two triple resonance MQ (multiple-quantum) 3D experiments were used which connect the H^{N}, N, CO and C^{α} resonances. The 3D MQ-HNCOCA experiment (Brutscher et al., 1995) connects the $H^{N}(i)$, N(i), CO(i − 1) and $C^{\alpha}(i-1)$ nuclei whereas in the 3D MQ-COHNCA experiment (Szyperski et al., 1995), magnetization is simultaneously transferred via one-bond scalar couplings to CO(i − 1) and $C^{\alpha}(i)$ and via a two-bond connectivity to $C^{\alpha}(i-1)$. In these reduced dimensionality experiments, four nuclei are frequency labelled in a three-dimensional spectrum. The two carbon frequencies are labelled in the same indirect dimension and the CO and C^{α} chemical shifts are then obtained from the separation (C^{α}) and the midpoint (CO) of the two peaks (see Figure 1). The midpoint frequency is confirmed by a 3D HNCO experiment. This approach allows the unambiguous correlation of the four resonances even in cases where the H^{N} and N resonances are degenerated. This information was completed by HN(CA)CO, HN(CA)CB and HN(COCA)CB triple resonance experiments and the sequential connectivities were built using the in-house program ALPS (Assignment of Labelled Protein Spectra, Morelle et al., 1995). With the exception of the HN(CA)CO and the HN(CA)CB experiments acquired at 800 MHz, all spectra were obtained at 600 MHz ^{1}H frequency and a triple labelled (^{2}H, ^{13}C, ^{15}N) sample was used. Aliphatic ^{1}H and ^{13}C sidechain assignment was performed using a double-labelled (^{13}C, ^{15}N) sample and the following experiments: H(CCO)NH-TOCSY, (H)C(CO)NH-TOCSY and a (H)CCH-TOCSY. For the latter, the protein sample was lyophilized and resuspended in D_2O. The (H)C(CO)NH-TOCSY spectrum also allowed unambiguous assignment of the $^{1}H^{N}$ and ^{15}N side chain resonances of Asn and Gln.

Data processing and peak picking were performed using FELIX program version 2000 (MSI Technologies).

Extent of assignments and data deposition

Unambiguous assignment of backbone resonances (H^{N}, N, C′, and C^{α}) was obtained for all residues (except A52, T53, and K137) Side chain assignment of the SiR-FP18 was accomplished for 97% of the aliphatic ^{13}C and 97% of the ^{1}H resonances.

The ^{1}H, ^{13}C, and ^{15}N backbone and side chain assignments of SiR-FP18 have been deposited with the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number BMRB-4985.

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