Journal of Biomolecular NMR, **18:** 365–366, 2000.
KLUWER/ESCOM

Letter to the Editor: Complete ^{1}H, ^{15}N and ^{13}C assignment of the functional domain of *Paracoccus denitrificans* cytochrome c_{552} in the oxidized state

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Received 11 July 2000; Accepted 12 September 2000

Key words: cytochromes, electron-transfer pathways, multidimensional NMR

Biological context

In the mitochondria of higher eukaryotes the electron transfer between complex III (ubiquinol:cytochrome *c* oxidoreductase) and complex IV (cytochrome *c* oxidase) is mediated by a single cytochrome *c* species. In bacteria, on the other hand, several *c*-type cytochromes are found due to the branching of the electron transport chains, requiring specific recognition and interaction between the electron transfer complexes. In the soil bacterium *Paracoccus denitrificans* the membrane-bound cytochrome c_{552} is the most likely mediator between complex III and IV (Berry and Trumpower, 1985).

Recently, a crystallographic study determined the structures of cytochrome c_{552} (*cyt* c_{552} in the following) from *P. denitrificans* both in the oxidized and reduced state (Harrenga et al., 2000). Even though the conformational differences appeared to be minor, a greater flexibility of the oxidized structure has been proposed. Similarily, a NMR study on another cytochrome *c* from *Saccharomyces cerevisiae* has also indicated a greater structural flexibility of the oxidized state based on hydrogen exchange data (Banci et al., 1997). Therefore, we are interested in studying the dynamic properties of both the reduced and the oxidized *cyt* c_{552} in solution. Previously, a soluble fragment of 10.5 kDa size, containing the functional domain of *cyt* c_{552} from *P. denitrificans* heterologously expressed in *E. coli* (Reincke et al., 1999), was used to obtain the resonance assignment of the reduced protein (Pristovšek et al., 2000). Here we present the first complete sequence-specific resonance assignment of *cyt* c_{552} in the paramagnetic oxidized state.

Methods and results

The soluble functional domain of *cyt* c_{552} was expressed and purified as described earlier (Pristovšek et al., 2000). The NMR samples were prepared in argon-purged 20 mM phosphate buffer (H_2O:D_2O = 95:5, v/v) at pH 5.8 with protein concentrations ranging between 1.5 and 4 mM. Potassium hexacyanoferrate(III) in small excess was used for oxidation. All NMR experiments were recorded at 298 K on Bruker DMX spectrometers operating at ^{1}H resonance frequencies of 499.87 and 600.13 MHz. All 3D experiments made use of pulsed field gradients for coherence selection and artifact suppression, and utilized gradient sensitivity enhancement schemes wherever appropriate (Muhandiram and Kay, 1994). Chemical shifts were referenced to internal DSS (Wishart et al., 1995) to ensure consistency among all spectra.

Due to the paramagnetic heme iron, the proton resonances in the oxidized state range from 32.43 ppm (Heme HMA) to −33.86 ppm (Met78 Hγ). Nevertheless, in contrast to the reduced form of *cyt* c_{552}, the oxidized protein shows less spectral dispersion in the amide region despite the paramagnetic center. Therefore, homonuclear and ^{15}N-edited NMR experiments, as described previously (Pristovšek et al.,

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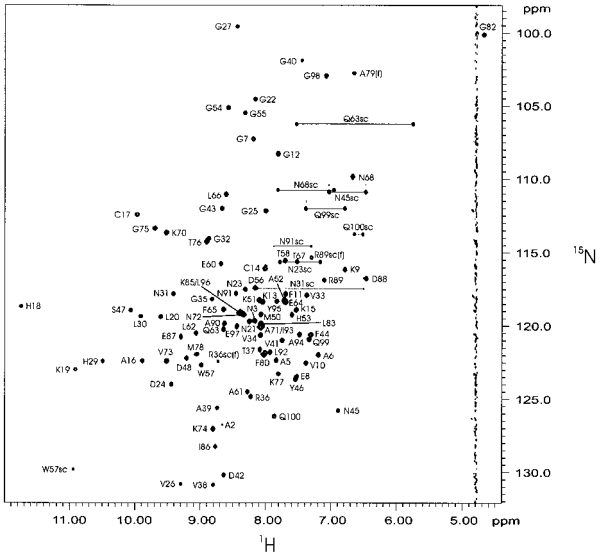

Figure 1. ^{1}H-^{15}N-HSQC spectrum (600 MHz, 298 K, pH 5.8) of the functional domain of *Paracoccus denitrificans* cytochrome c_{552} in the oxidized state. Signals from amino acid side-chains are denoted with 'sc'. The HN peaks from the Arg side-chains and the Ala79 backbone are folded (f) in the ^{15}N dimension.

2000), were not sufficient to obtain the sequential resonance assignment using the classical assignment strategy through NOE connectivities. Instead, triple-resonance experiments were performed on the double-enriched sample. The HNCACB and CC(CO)NH-TOCSY (spinlock time 21.1 ms) experiments were used to obtain the aliphatic carbon resonances. From the (HCA)CO(CA)NH experiment (Löhr and Rüterjans, 1995) the carbonyl resonances were determined. Additionally, ^{1}H-^{13}C-HSQC as well as ^{13}C-edited HCCH-TOCSY (spinlock time 21.1 ms) and NOESY-HSQC (mixing time 75 ms) spectra provided valuable information about carbon resonances of both the protein and the heme moiety.

Despite the presence of the paramagnetic center, only 4 proton resonances (at 27.65, −16.33, −24.30 and −33.86 ppm), which display extreme line-broadening and unusually fast relaxation due to their close proximity to the iron, could not be assigned by the above-described procedure. Therefore, in addition to ^{1}H-^{1}H NOESY spectra with rather short relaxation and mixing times of 0.4 s and 30 ms, respectively, a series of 1D NOE difference experiments with selective saturation (100 ms irradiation time) of the unidentified proton resonances, as previously applied for other paramagnetic proteins (Banci et al., 1989; Satterlee and Erman, 1991), were performed to complete the ^{1}H resonance assignment of *cyt* c_{552}.

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C backbone and side-chain resonances of *cyt* c_{552} in the oxidized state have been assigned (at pH 5.8 and 298 K), except for several side-chain carbonyl carbon atoms and some carbon atoms in the immediate vicinity of the paramagnetic iron, i.e., in the heme group and in the side-chains of the axial ligands. Aside from the strong ring-current effects due to the heme moiety and the aromatic protein side-chains, which lead to many unusual chemical shift values such as 4.67 ppm for the Gly82 amide proton (Figure 1, upper right corner; at 4.50 ppm in the reduced state), the paramagnetic iron atom induces large chemical shift changes in neighboring nuclei like the heme methyl protons HMA, HMB, HMC and HMD at 32.43, 13.12, 32.27 and 16.57 ppm, respectively. The sequence-specific resonance assignment has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database under accession number BMRB-4777.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft SFB 472. P.P. thankfully acknowledges the receipt of a Humboldt fellowship.

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