Journal of Biomolecular NMR, **16:** 353–354, 2000.
KLUWER/ESCOM
© 2000 *Kluwer Academic Publishers. Printed in the Netherlands.*

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 reduced state

Primož Pristovšek*, Christian Lücke[a], Britta Reincke[a], Frank Löhr[a], Bernd Ludwig[b] & Heinz Rüterjans[a,]**
[a]*Institute of Biophysical Chemistry, J.W. Goethe University of Frankfurt, Marie-Curie-Straße 9, D-60439 Frankfurt am Main, Germany;* [b]*Institute of Biochemistry, Molecular Genetics, J.W. Goethe University of Frankfurt, Germany*

Received 9 December 1999; Accepted 24 January 2000

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

Biological context

Cytochrome c_{552} is a well-established electron mediator between the last two complexes of the *Paracoccus denitrificans* respiratory chain, the cytochrome bc_1 complex and the aa_3-type cytochrome *c* oxidase. It contains a covalently bound heme moiety, with the iron atom alternating between oxidation states 2+ (diamagnetic) and 3+ (paramagnetic) during its redox cycle. The iron is octahedrally coordinated by six ligands, the axial ones being a histidine and a methionine.

The structural aspects of cytochromes *c* have been extensively studied. Several NMR solution structures of different cytochromes *c* are known to date (e.g.: Baistrocchi et al., 1996; Qi et al., 1996; Hasegawa et al., 1998). Until now, however, ^{13}C/^{15}N-enrichment has been reported only for cytochrome c_2 (Caffrey et al., 1994) and cytochrome c' from *Rhodobacter capsulatus* (Caffrey et al., 1995), and *Thiobacillus versutus* ferrocytochrome c_{550} (Ubbink et al., 1996).

We are interested in studying the molecular interactions and electron-transfer reactions of *P. denitrificans* cytochrome c_{552} (*cyt* c_{552} in the following) with its redox partners. However, unlike in mitochondria, this *cyt* c_{552} is a membrane-bound protein. We have recently been able to express a soluble fragment (10.5 kDa) containing the functional domain of this cytochrome in *E. coli* (Reincke et al., 1999). This fragment, which shows electron-transfer activity, is obtained in yields high enough to allow isotopic labelling. In order to determine the solution structure of *cyt* c_{552}, we have produced both ^{15}N- and ^{13}C/^{15}N-labelled protein. This has now led to the first complete sequence-specific assignment of cytochrome c_{552} in the reduced state.

*Present address: National Institute of Chemistry, Ljubljana, Slovenia.
**To whom correspondence should be addressed. E-mail: hruet@bpc.uni-frankfurt.de

Methods and results

The soluble functional domain of *cyt* c_{552} was expressed and purified as described earlier (Reincke et al., 1999). For ^{13}C- and/or ^{15}N-labelling, the cells were grown in several 100 ml batches of Bioexpress-1000 medium (Cambridge Isotope Laboratories) in order to achieve maximum protein yield.

The NMR samples were prepared in argon-purged phosphate buffer (20 mM, pH 6.0; H_2O:D_2O = 90:10, v/v) at protein concentrations ranging between 1.5 and 4 mM. Sodium dithionite in small excess was used for reduction, which caused the pH in the solution to drop by 0.5 units. 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). Quadrature detection in the indirectly detected dimensions was obtained either by the States-TPPI or by the echo/antiecho method. Chemical shifts were referenced to internal DSS (Wishart et al., 1995) to ensure

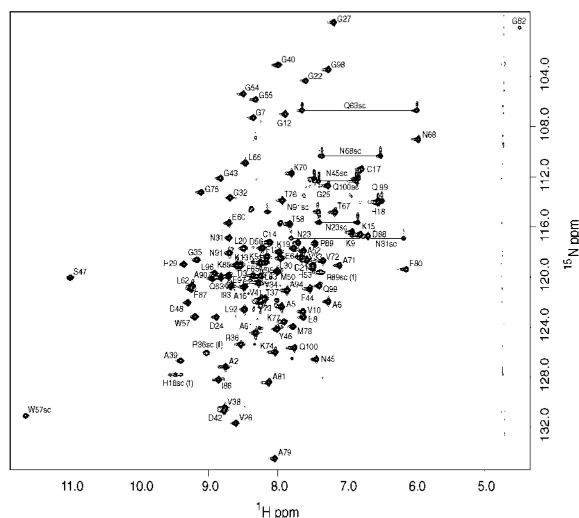

Figure 1. ^{1}H-^{15}N-HSQC spectrum (^{1}H resonance frequency 600.13 MHz, 298 K, pH 5.5) of the functional domain of *Paracoccus denitrificans* cytochrome c_{552} in the reduced state. Signals from side-chain amide groups are denoted with 'sc'; in case of Arg and His residues these peaks are folded (f).

consistency among all spectra. The spectral data were processed using the Bruker XWIN-NMR 1.3 software package; peak-picking and data analysis of the transformed spectra were performed using AURELIA 2.5.9 (Bruker) and FELIX 97 (MSI). The final 3D matrices typically consisted of $1024 \times 128 \times 128$ or $1024 \times 128 \times 256$ real data points.

The sequence-specific ^{1}H and ^{15}N assignments were almost completely determined from homonuclear 2D (TOCSY and NOESY) and ^{15}N-edited multidimensional (HSQC, HTQC, TOCSY-HSQC and NOESY-HSQC) spectra, using the classical assignment strategy through NOE connectivities. The TOCSY experiments were performed with spinlock times of either 80 ms or 5 ms (to obtain COSY-type information with less spectral overlap). For the NOESY experiments mixing times of 50 ms and 80 ms were used.

In addition, in order to confirm and complete the assignments, ^{13}C-edited experiments were performed on the double-enriched sample. The HNCACB and CC(CO)NH-TOCSY (spinlock time 19.8 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. Finally, ^{1}H-^{13}C-HSQC as well as ^{13}C-edited NOESY-HSQC spectra for aliphatic (mixing time 75 ms) and aromatic (mixing time 120 ms) protons gave additional valuable information about carbon resonances of both the protein and the heme moiety.

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 reduced state have been assigned (at pH 5.5 and 298 K), except for several side-chain carbonyl carbon atoms. Due to ring-current effects, especially from the heme moiety, many very unusual chemical shift values have been observed, such as 4.50 ppm for the Gly82 amide proton, as seen in the upper right corner of the ^{1}H-^{15}N-HSQC spectrum (Figure 1). The sequence-specific assignment has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database under accession number BMRB-4471.

Acknowledgements

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

References

Baistrocchi, P., Banci, L., Bertini, I., Turano, P., Bren, K.L. and Gray, H.B. (1996) *Biochemistry*, **35**, 13788–13796.

Caffrey, M., Brutscher, B., Simorre, J.P., Fitch, J., Cusanovich, M. and Marion, D. (1994) *Eur. J. Biochem.*, **221**, 63–75.

Caffrey, M., Simorre, J.P., Brutscher, B., Fitch, J., Cusanovich, M. and Marion, D. (1995) *Biochemistry*, **34**, 5904–5912.

Hasegawa, J., Yoshida, T., Yamazaki, T., Sambongi, Y., Yu, Y., Igarashi, Y., Kodama, T., Yamazaki, K., Kyogoku, Y. and Kobayashi, Y. (1998) *Biochemistry*, **37**, 9641–9649.

Löhr, F. and Rüterjans, H. (1995) *J. Biomol. NMR*, **6**, 189–197.

Muhandiram, D.R. and Kay, L.E. (1994) *J. Magn. Reson.*, **B103**, 203–216.

Qi, P.X., Beckmann, R.A. and Wand, A.J. (1996) *Biochemistry*, **35**, 12275–12286.

Reincke, B., Thöny-Meyer, L., Dannehl, C., Odenwald, A., Aidim, M., Witt, H., Rüterjans, H. and Ludwig, B. (1999) *Biochim. Biophys. Acta*, **1411**, 114–120.

Ubbink, M., Pfuhl, M., Van der Oost, J., Berg, A. and Canters, G.W. (1996) *Protein Sci.*, **5**, 2494–2505.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.