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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N signals of bovine adrenodoxin

R. Weiß[a], L. Brachais[b], F. Löhr[a], J. Hartleib[a], R. Bernhardt[a,*] & H. Rüterjans[a,**]
[a]*Institut für Biophysikalische Chemie, Johann Wolfgang Goethe-Universität, Biozentrum N230, Marie-Curie-Str. 9, D-60439 Frankfurt am Main, Germany;* [b]*LRP-UMR C7581 CNRS, 2-8 rue Henry Dunant, F-94329 Thiais, France*

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Biological context

Adreno-Ferredoxin (Adrenodoxin/Adx) is a small [2Fe2S] ferredoxin. The 128 residue protein is involved in the electron transfer from the flavoenzyme NADPH-adrenodoxin-reductase to cytochrome P-450_{ssc} respective cytochrome P-$450_{11\beta}$. It is a member of the mitochondria electron transport chain responsible for the production of steroid hormones in mammals. It is located in brain (Stormer et al., 1979) and in several tissues including the adrenals (Kimura and Suzuki, 1965), placenta (Cupp et al., 1988), gonads (Kimura, 1968), and in low amount in liver (Okuda and Atsuta, 1978; Saarem et al., 1981; Hiwatashi et al., 1986).

Methods and results

1 to 5 mM oxidized Adrenodoxin samples were obtained from *E. coli* and purified as described elsewhere (Uhlmann et al., 1992). For ^{15}N labelling of samples, $^{15}NH_4Cl$ was used and for ^{13}C labelling, both $^{13}C_6$ glucose and $^{13}C_3$ glycerol were used as sole ^{15}N or ^{13}C source in the growth medium. The purity of the protein was analysed by measuring $A_{414}/A_{280} > 0.9$ in Tris/HCl (50 mM, pH = 7.4) buffer. 5% D_2O was added to the solution providing the lock signal. 5% of D_6 glycerol was added into the NMR tube to inhibit the degradation of Adx.

NMR experiments were recorded on DMX500 and DMX600 Avance Bruker spectrometers equipped with triple resonance probeheads and PFG accessory.

The assignment of the protein backbone was based on eight different experiments: HNCA, HN(CO)CA, H(N)CA,CO, HNCACB, HNCO, HBHA(CO)NH, HCACO, (HCA)CO(CA)NH. The advantage in combining all these experiments is the low risk of wrong assignment since each chemical shift value can usually be obtained from several experiments in a different correlation. The side chain assignments were achieved using TOCSY-^{15}N-HSQC, HNHB, HCCH-TOCSY and CCC(O)NH-TOCSY experiments. $^{15}NH_n$ resonances of Asn, Gln, Arg, were observed in a conventional 2D-(^{15}N)-HSQC experiment.

Extent of assignments and data deposition

Most of ^{15}N, ^{13}C and ^{1}H resonances could be assigned using various 3D heteronuclear experiments. Eventually, the resonances of F43, G44, A45,C46, E47, G48, T49, L50, A51, C52, S53, T54, C55, H56, L90, G91, C92, Q93 and I94 could not be identified in the spectra because of the paramagnetic character of the [2Fe2S] cluster, since in the oxidized state, both irons in the iron-sulfur cluster are high-spin Fe(III) and are antiferromagnetically coupled in the ground state (S=0). This accounts for the paramagnetic effects observed in NMR spectra of bovine Adx at physiological temperatures, interfering with nuclear Overhauser effect (NOE) studies and causing broad line widths. Further chemical shifts could not be assigned because of the degradation of the C-terminal amino acid residues. Also the resonances of the first three serine residues could not be found due to degradation.

*Present address: FR 12.4 Biochemie, Universität des Saarlandes, P.O. Box 151150, D-66041 Saarbrücken, Germany.
**To whom correspondence should be addressed. E-mail: hruet@bpc.uni-frankfurt.de

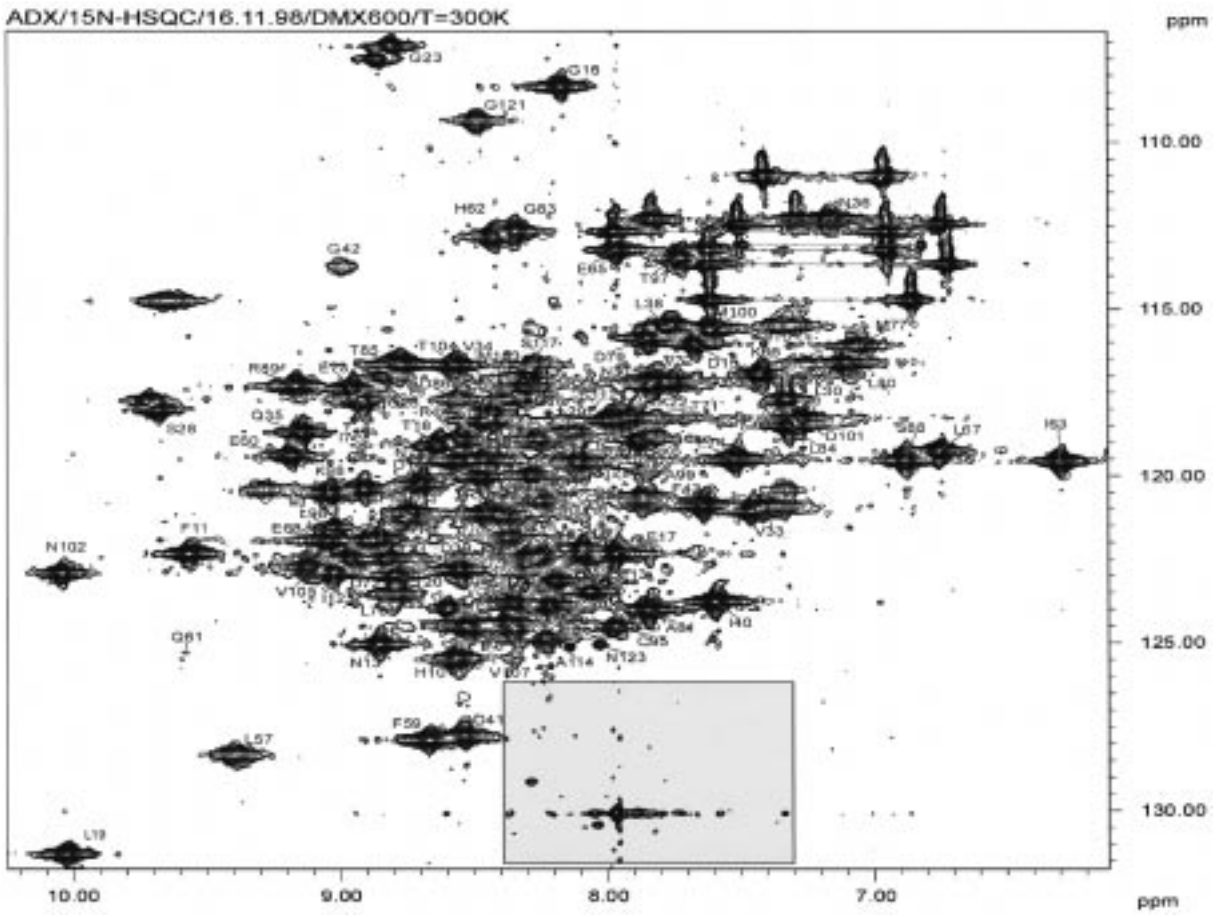

Figure 1. 2D-(^{1}H-^{15}N)-HSQC experiment of Adx in Tris/HCl (50 mM, pH = 7.4) buffer. The solution contained 5% D_2O for field locking and 5% of D_6 glycerol to inhibit the degradation of Adx. Sequential assignments are reported in the spectrum. Pairs of peaks connected by a horizontal line represent Asn and Gln side chain $^{15}NH_2$ resonances. The numerous narrow peaks appearing in the marked area are characteristic for the adrenodoxin degradation.

From a chemical shift index (not shown) the secondary structure of Adx was derived. These results suggest that the solution structure of Adx is quite similar to its recently published X-ray structure (Müller et al., 1998). In particular, Pro108 seems to be an essential residue for the correct folding of the protein.

The data deposition number is BMRB-4566. The data table, as submitted, can be viewed from the BMRB 'hidden directory' using the following URL: http://www.bmrb.wisc.edu/elec_dep/author_view/assign_notes/W2sx7Y.pd

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