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Assignment of ^{1}H, ^{13}C, and ^{15}N signals of reduced *Clostridium pasteurianum* rubredoxin: Oxidation state-dependent changes in chemical shifts and relaxation rates*

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Biological context

Rubredoxin from *Clostridium pasteurianum* (Rdx) is a paramagnetic FeS_4 iron–sulfur protein. Its biological relevance has been described previously (Volkman et al., this issue), along with diamagnetic resonance assignments of oxidized [Fe(III)] Rdx. Here we report the diamagnetic ^{1}H, ^{15}N and ^{13}C resonance assignments for Fe(II) Rdx and examine their dependence on the oxidation state of Rdx.

Methods and Results

NMR samples of reduced [*U*-^{15}N]- and [*U*-^{15}N,^{13}C]-Rdx were prepared as previously described (Xia et al., in preparation). All samples contained ~4–6 mM rubredoxin in 50 mM phosphate buffer, pH 6.0. NMR experiments were recorded as described previously (Volkman et al., 1997) with the parameters shown in Table 1 at 25 °C on a Bruker DMX500 spectrometer equipped with a triple-resonance $^{1}H/^{13}C/^{15}N$ probe and triple-axis pulsed field gradient capabilities.

Data processing was performed with FELIX95 (Molecular Simulations, San Diego, CA, U.S.A.) as previously described (Volkman et al., 1997). All ^{1}H dimensions were referenced to internal DSS (2,2-dimethyl-2-silapentane-5-sulfonate). ^{13}C and ^{15}N dimensions were indirectly referenced to DSS as previously described (Wishart et al., 1995). Chemical shifts for all cross peaks were tabulated and assigned as previously described (Volkman et al., 1997).

Extent of assignments and data deposition

Sequence-specific assignments for reduced rubredoxin were obtained from the HNCACB and CCONH data. Hyperfine shifts affect 12 backbone ^{15}N signals arising from the two CXXCGX motifs that provide covalent bonds to the iron (Xia et al., in preparation). No signals from these 12 residues (C6-Y11 and C39-V44; see the gray letters in Fig. 1) are observed in any of the 2D or 3D NMR spectra. In reduced Rdx, correlations to all other backbone amides were seen, whereas in oxidized Rdx, 10 of these NH correlations were missing in the HSQC spectrum (Fig. 1). Two of the HSQC peaks for reduced Rdx were not assigned conclusively from triple resonance data, but G45(t) has a distinctive ^{15}N shift which suggests that it is a glycine. Taking into account the separate identification of the 12 hyperfine-shifted signals (Xia et al., in preparation), the remaining peak likely corresponds to I12. Including the two tentatively assigned HSQC peaks, the reduced form shows signals from K2-T5, I12-V38, and G45-E54. The largest ^{1}H and ^{15}N chemical shift differences between Fe(II) Rdx and Fe(III) Rdx occur in the C-terminal residues and for residues adjacent to the hyperfine-shifted CXXCGX regions (Fig. 1). This is consistent with the results of energy minimization calculations on rubredoxin, which predict

*These data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4050.

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Abbreviations: Rdx, recombinant *Clostridium pasteurianum* rubredoxin produced in *Escherichia coli* and reconstituted with iron.

TABLE 1
PARAMETERS FOR NMR EXPERIMENTS USED IN THE $^{1}H/^{15}N/^{13}C$ ASSIGNMENTS OF REDUCED RUBREDOXIN

Experiment	^{1}H			D2			D3			Matrix dimensions[c]	Mixing time[d]	BMRB[e]
	SF (MHz)[a]	SW (Hz)	N*[b]	Nucleus	SW (Hz)	N*	Nucleus	SW (Hz)	N*			
HNCO[f]	500.13	8333.33	1K	$^{13}C'$	2000	60	^{15}N	1666.67	36	512×256×128		1
HNCACB[f]	500.13	8333.33	1K	$^{13}C^{\alpha\beta}$	8333.33	60	^{15}N	1666.67	40	512×256×128		2
C(CO)NH[f]	500.13	8333.33	1K	^{13}C	8771.95	64	^{15}N	1666.67	40	512×256×128		
HCCH-TOCSY[g]	500.13	4166.67	512	^{1}H	4166.67	128	^{13}C	4000	64	512×512×128	19	
$^{15}N/^{1}H$ HSQC	500.13	8333.33	1K	^{15}N	1666.67	200				512×512		85
$^{13}C/^{1}H$ CT-HSQC	500.13	7716.05	1K	^{13}C	4000	114				1024×512		

[a] ^{1}H frequency.
[b] Number of complex points collected in this indirect dimension.
[c] Final processed matrix size.
[d] Time in ms for isotropic mixing times.
[e] BMRB pulse program library accession number.
[f] Experiments used for backbone assignments.
[g] To complete side-chain assignments.

that oxidation state-dependent structural changes will be localized to these regions (Shenoy and Ichiye, 1993).

According to the crystal structure (Watenpaugh et al., 1980), the line-broadened signals are within 8.5 Å and 11 Å, respectively, from the iron for reduced and oxidized Rdx. The patterns of line broadening for the ^{15}N and $^{1}H^{N}$ resonances in the two oxidation states are consistent with the change in total electron spin from S = 5/2 in the oxidized form to S = 2 for reduced rubredoxin, with fewer resonances experiencing extreme paramagnetic broadening in the reduced state. Relaxation due to the dipolar field of the thermally averaged electronic spin (Curie spin) is likely to play a dominant role in the paramagnetic relaxation. Chemical shift assignments for reduced Rdx have been deposited at BioMagResBank under the accession number given in the first footnote on page 417; pulse sequences have been deposited under the accession numbers given in Table 1.

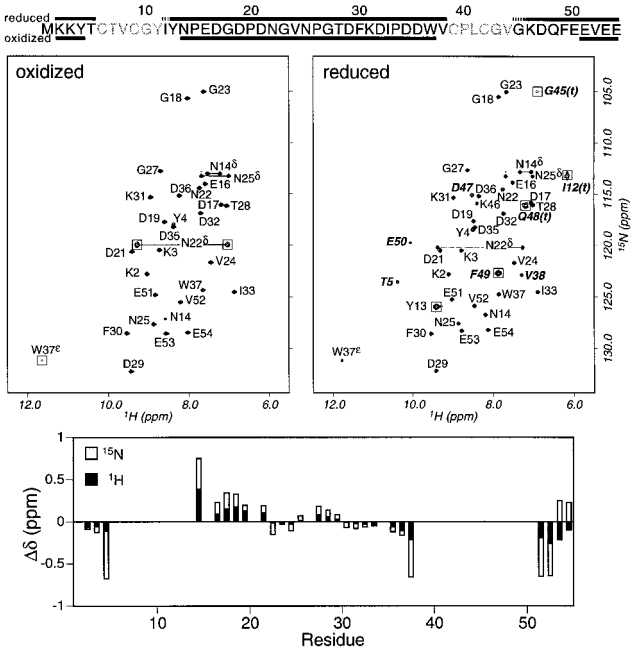

Fig. 1. Resonance assignments of oxidized and reduced *C. pasteurianum* rubredoxin. The amino acid sequence is shown with bars above and below indicating residue assignments in reduced and oxidized Rdx, respectively. Residues in gray surround the iron and contain hyperfine-shifted resonances not observed in the diamagnetic spectral regions. Labels indicate assignments (residue type and sequence number) for the ^{1}H-^{15}N HSQC spectra of oxidized and reduced Rdx in 90% H_2O, pH 6.0 at 298 K. Contours are drawn at the same relative level in the two spectra, and peaks that were observed at lower contour levels are indicated by boxes and shown with a base contour level of 0.2 times that of the main spectrum. Residues that appear in the spectrum of reduced Rdx but not in that of oxidized Rdx are labeled in bold italics. Chemical shift differences for amide $^{1}H^{N}$ and ^{15}N resonances assigned in both oxidation states are plotted as a function of their position in the amino acid sequence.

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