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Letter to the Editor: Backbone sequential resonance assignments of yeast iso-2 cytochrome *c*, reduced and oxidized forms

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Biological context

Iso-2 cytochrome *c* is a small globular heme protein found in the intermembrane of yeast (*Saccharomyces cerevisiae*) mitochondrion. Its main biological function is to transfer electrons in the respiratory chain.

Expression of the homologous iso-1 cytochrome *c* in *E. coli* has been made possible by methods involving the recombinant expression of the cytochrome *c* gene and the heme lyase gene (Pollock et al., 1998). A similar approach was taken for iso-2 cytochrome *c*.

We report the NMR assignments of reduced and oxidized $^{13}C/^{15}N$ double-labeled K72A iso-2 cytochrome *c*. This variant of iso-2 cytochrome *c* will be used as our 'wild-type' protein for future hydrogen exchange studies. The K72A mutation was introduced to prevent coordination of Lys72 to the heme iron which generates the inactive alkaline form of cytochrome *c* (Pollock et al., 1998; Delange et al., 1970; Pearce et al., 1989; Rosell et al., 1997).

Methods and experiments

The construction of an expression plasmid pER1CYC7 for yeast iso-2 cytochrome *c* in *E. coli*, consisted of replacing the CYC1 gene (iso-1 cytochrome *c*) of pBTR2 (Rosell & Mauk, unpublished; Pollock et al., 1998) with an insertion of a 0.522 kb Nco1, Bam H1 fragment containing the CYC7 gene (iso-2 cytochrome *c*) obtained from pEMBLYe30CYC7 (McGee et al., 1996). Appropriate restriction sites and the K72A mutation were introduced by site-directed mutagenesis. The pER1CYC7 plasmid was used to transform the protease deficient *E. coli* strain, BL21 (Stratagene). One liter cultures were grown as described (Cai et al., 1998) resulting in 8–15 mg/l of double-labeled protein. The NMR samples were prepared by dissolving protein in 50 mM Sodium Phosphate, 5%^2H_2O at pH 6.0.

Sequential assignments of reduced and oxidized K72A iso-2 cytochrome *c* were made using triple resonance methods at 20 °C. The NMR data was collected using a Bruker AMX2-500 spectrometer. ^1H, ^{13}C and ^{15}N chemical shifts were referenced to DSS according to the IUPAC recommendation (Markley et al., 1998). The sequential connectivities were made using the C(CO)NH and HNCACB pair of experiments, and were confirmed using HNCO and HN(CA)CO experiments. The C(CO)NH experiment allowed almost complete side chain ^{13}C assignments. Assignments for backbone Hα and sidechain Hβ resonances were obtained using the HBHA(CO)NH experiment.

Extent of assignments and data deposition

Backbone and side chain ^{13}C and ^{15}N sequential resonance assignments (HN, N, Cα, Hα, Cβ, Hβ, Cγ, Cδ, Cε, and C^O) were obtained for 104 and 105 of the 112 amino acid residues of oxidized and reduced K72A iso-2 cytochrome *c* (Figures 1A and 1B, respectively). For the oxidized form, partial assignments are available for 7 of the 8 remaining residues: K(−8), P(−1), P25, P30, P71, P76, G84. For the reduced form, 7 of the remaining residues are partially assigned: A(−9), P(−1), P25, P30, P71, P76, G84.

Comparison of the chemical shifts of the homologous iso-1 cytochrome *c* (Fetrow & Baxter, 1999;

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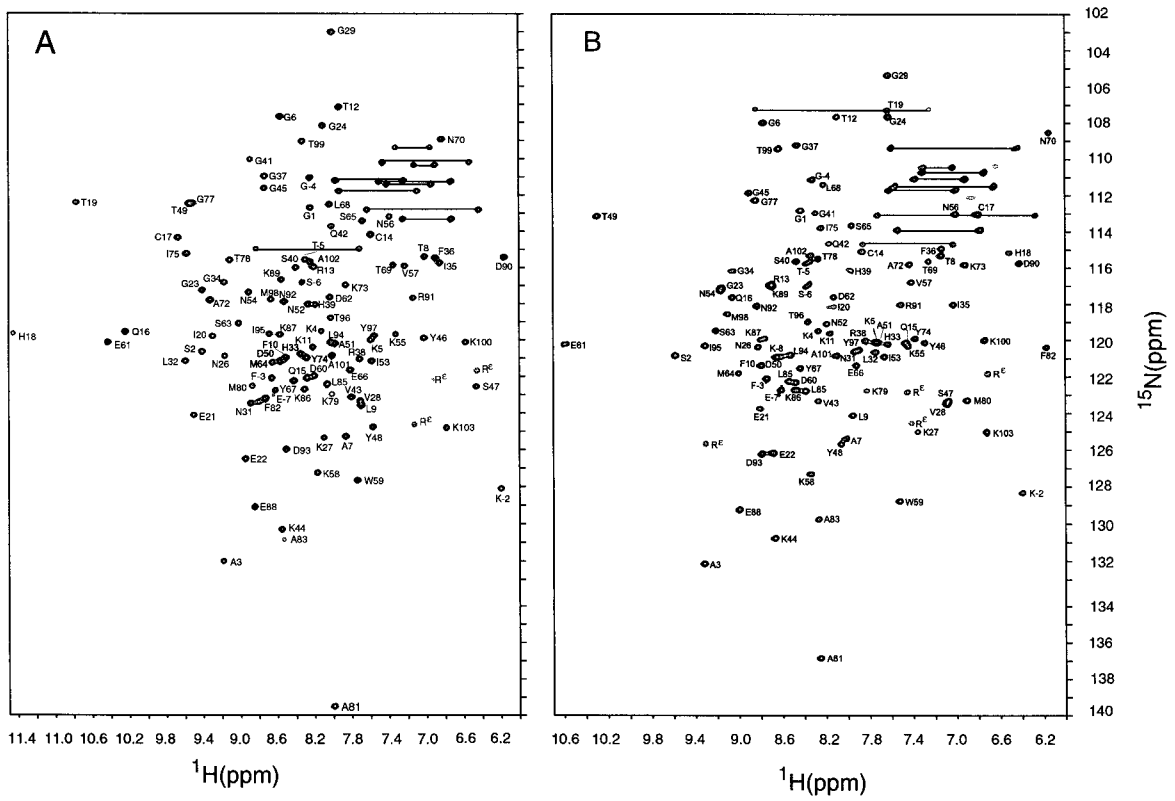

Figure 1. Summary of the backbone assignments reported herein as illustrated by 2D ^{1}H–^{15}N HSQC spectrum of oxidized (A) and reduced (B) K72A yeast iso-2 cytochrome *c*. Peaks are numbered according to the backbone amino acid sequence. Sidechain amide groups are indicated by solid lines parallel to the ^{1}H axis. R^{ε} indicates arginine sidechain N^{ε}-H that is folded into the spectrum.

Szabo et al., 2000) to those of iso-2 cytochrome *c* reveals good agreement between the chemical shifts for those amino acid residues that iso-1 and iso-2 have in common. The iso-2 cytochrome *c* assignments have been deposited with BioMagResBank under accession 5003 (reduced) and accession 5005 (oxidized).

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