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Letter to the Editor: Sequence-specific resonance assignments for the NADP(H)-binding component (domain III) of proton-translocating transhydrogenase from *Rhodospirillum rubrum*

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Biological context

Transhydrogenase is a proton pump, found in the inner membrane of animal mitochondria, and the cytoplasmic membrane of bacteria. It has a tripartite structure. Domains I and III protrude from the membrane (on the cytoplasmic side in bacteria, and on the matrix side in mitochondria). The domain II component spans the membrane, and serves as a channel for proton conduction. Transhydrogenase couples the transfer of reducing equivalents (hydride ion equivalents) between NAD(H) and NADP(H) to the translocation of protons across the membrane (reviewed by Jackson et al., 1998),

$$n\mathrm{H}^+_{\mathrm{out}} + \mathrm{NADH} + \mathrm{NADP}^+ \Leftrightarrow$$
$$n\mathrm{H}^+_{\mathrm{in}} + \mathrm{NAD}^+ + \mathrm{NADPH} \qquad (1)$$

where n is probably 1.0. Hydride transfer between NAD(H) bound to domain I, and NADP(H) bound to domain III, is direct and proceeds without involvement of intermediate redox reactions, implying that the C4 atoms of the nicotinamide rings of the two nucleotides must be brought into close apposition during catalysis. Under physiological conditions the equilibrium is driven from left to right (Equation 1) by the Δp generated by the primary proton pumps of respiration or photosynthesis. The function of transhydrogenase in bacteria is to provide NADPH for amino acid biosynthesis and for the reduction of glutathione. In mitochondria, it is again required in the production of reduced glutathione, and it may have a role in the regulation of flux through the tricarboxylic acid cycle.

In different species, the three domains of transhydrogenase are distributed across one, two or three polypeptide chains. For example, in the photosynthetic bacterium *Rhodospirillum rubrum*, there are three polypeptides, PntAA (comprising domain I), PntAB (comprising domain IIa) and PntB (comprising domains IIa and III). In *Escherichia coli* there are two independent polypeptides, the first consisting of domains I–IIa, and the second of domains IIb–III. In bovine mitochondria and in the parasitic protozoan *Eimeria tenella* there is a single polypeptide, with the domains arranged in the orders I-II-III and IIb-III-I-IIa, respectively. The isolated domains I and III of transhydrogenase from a number of organisms have been cloned and expressed, for example, domain I protein (Diggle et al., 1995) and domain III protein (Diggle et al., 1996) from *R. rubrum*. In solution, mixtures of expressed domain I and domain III (even from enzymes of different species) are catalytically active (Diggle et al., 1996), and hence provide a convenient system in which to investigate the relationship between the structure of the domains I and III and the mechanism of hydride transfer. We here report the sequence-specific assignments for isolated recombinant domain III from *R. rubrum*. Calculation of the structure of this isolated domain III-NADP^+ complex is underway.

Methods and results

Transhydrogenase domain III from *R. rubrum* (molecular weight 21.5 kDa, 203 amino acids) was cloned, expressed and purified as described in Diggle et al. (1996), except that the protein was cloned into pET11c

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vector and *E. coli* BL21(DE3) host cells were used for the expression. During the preparation procedure all the nucleotide bound to domain III was converted to $NADP^+$ by mixing domain III with domain I (in a molar ratio of 1:20 with domain III) and 50 μM acetylpyridine adenine dinucleotide ($AcPAD^+$) followed by subsequent chromatographic isolation of the domain III protein. Protein solutions of the U-^{15}N- or U-^{13}C,^{15}N-labelled domain III-$NADP^+$ complex were prepared at 800 μM in 20 mM Hepes, pH 7.2, 0.01% (w/v) NaN_3, 20 μM AEBSF protease inhibitor (ICN Biomedicals Inc.), 2 μM excess $NADP^+$, 90% H_2O/10% D_2O. All experiments were performed on a three-channel Varian Unityplus 600 spectrometer at 30 °C, using a 5 mm triple resonance ^{1}H/^{13}C/^{15}N Z-gradient probe.

Main-chain $^1H^N$, ^{15}N, $^{13}C^\alpha$ and side-chain $^{13}C^\beta$ resonances were assigned using HNCACB and CBCA(CO)NNH (Muhandiram and Kay, 1994) and Cbd-HNCA (Matsuo et al., 1996) experiments to establish segments of sequential connectivity. Main-chain $^1H^\alpha$ and $^{13}C'$ assignments were made using CBCACO(CA)HA (Kay, 1993), HNCO (Muhandiram and Kay, 1994), HBHA(CBCA)(CO)NNH (Grzesiek and Bax, 1993) and HNHA (Kuboniwa et al., 1994). Where appropriate, selective carbon decoupling was achieved using WURST-2 adiabatic decoupling schemes (Matsuo et al., 1996).

Extent of assignments and data deposition

Sequence-specific assignments ($^1H^N$, ^{15}N, $^{13}C^\alpha$, $^1H^\alpha$, $^{13}C^\beta$, $^{13}C'$) for recombinant domain III from *R. rubrum* have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database (accession number 4236). Figure 1 shows an annotated ^{1}H-^{15}N HSQC spectrum of domain III. Virtually complete resonance assignments have been made for the protein between residues F13 and N203 (98% of assignments made, with only residue Y147 and the proline residues having less than four resonances assigned). At the N-terminus no data are given for residues M1–G12 (only tentative assignments have been made). Based upon the alignment of transhydrogenase sequences from different species this N-terminal sequence appears to form part of a linker region between domains IIb and III.

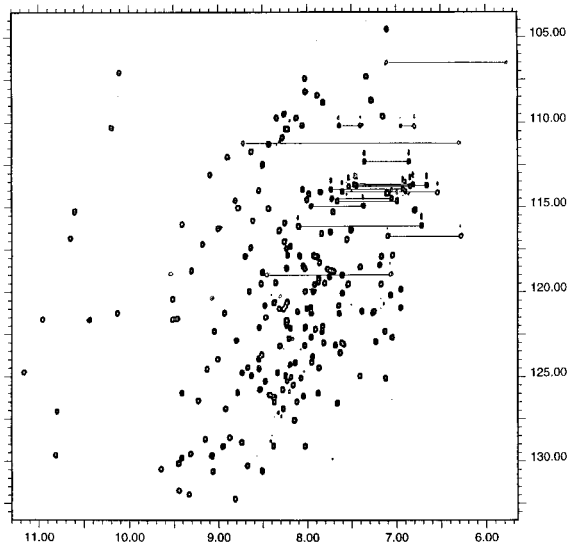

Figure 1. Sensitivity enhanced 2D ^{1}H-^{15}N HSQC spectrum of 800 μM ^{15}N-labeled transhydrogenase domain III from *R. rubrum* at 30 °C and pH 7.2. Side chain NH_2 resonances of asparagine and glutamine are connected by bars.

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