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Letter to the Editor: Sequential assignment and secondary structure analysis of the NADP(H)-binding domain of *Escherichia coli* transhydrogenase

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Biological context

Nicotinamide nucleotide transhydrogenase (EC 1.6.1.1) is a membrane-bound enzyme that links the energy stored in the electrochemical gradient with the redox state of NADP(H). The reduction of $NADP^+$ by NADH is coupled to proton translocation across the membrane. The enzyme consists of three domains, domain I and III that bind NAD(H) and NADP(H), respectively, and the membrane spanning domain II, through which protons are translocated. In *Escherichia coli*, domain I comprises residues α1-α404 of the α-subunit, and domain III residues β286–β462 of the β-subunit.

Little structural information is known for transhydrogenase. A predicted model of domain I has been presented, and it was suggested that NAD(H) binds to a classical dinucleotide binding domain (Fjellström et al., 1995). The structure of alanine dehydrogenase, a homologue to domain I of transhydrogenase, has recently been determined (Baker et al., 1998). Domain III, on the other hand, shows no significant sequence similarity with sequences in the databases, and the putative dinucleotide binding site is less obvious. Recently, the structure of the NADP(H)-binding site was predicted (Fjellström et al., 1999) and a secondary structure model of domain III of *R. rubrum* transhydrogenase based on NMR data was presented (Quirk et al., 1999).

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Methods and results

The C-terminal 177 residues of the protein (β286–β462) were subcloned in a T7 expression system following an N-terminal Met-$(His)_6$-Ser-Ser-tag, expressed in BL21(DE3) cells, purified as previously described (Fjellström et al., 1997). The protein behaves as a monomer in size exclusion chromatography and dynamic light scattering experiments (Johansson et al., unpublished results). The protein was uniformly labelled using ^{15}N ammonium chloride and/or ^{13}C glucose, and specifically ^{15}N-labelled using ^{15}N-lysine, ^{15}N-valine or ^{15}N-alanine. Deuterated protein was obtained by growing cells in 99% D_2O, using either ^1H glucose or ^2H glycerol as carbon source.

NMR samples contained 0.9 to 1.5 mM protein with bound $NADP^+$ in 10 mM phosphate and 100 mM NaCl at pH 7.0. NMR experiments were performed at the Swedish NMR Center on 600 or 800 MHz (NOESY experiments) Varian Inova spectrometers at 25 °C. Relaxation experiments were performed as described (Farrow et al., 1994). T_1 and T_2 relaxation times of 66 isolated peaks spread over the sequence were used to determine $\tau_c = 14.2 \pm 2.2$ ns ($T_1/T_2 = 20.4 \pm 5.7$) using the Modelfree software package (Mandel et al., 1995).

CT-HNCA, CT-HN(CO)CA and HNCO spectra were collected essentially as described (Shan et al., 1996; Yamazaki et al., 1994). ^{15}N TOCSY-HSQC, ^{15}N NOESY-HSQC and HCACO spectra were also used in the sequential assignment. DSS was used to reference ^1H spectra. ^{15}N and ^{13}C chemical shifts were referenced indirectly by multiplying the ^1H ref-

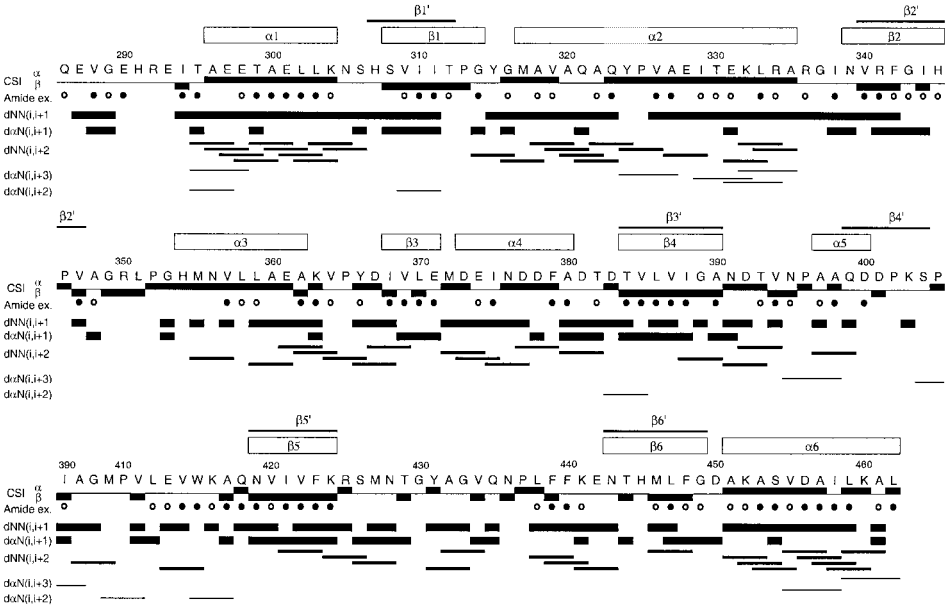

Figure 1. Summary of secondary structure, chemical shift index (integer value of (diff (C^{α}) + diff (C')-5× diff(H^{α})) divided by 3, α-helix positive, β-strand negative), short-range NOEs and amide proton exchange (open circles, ^{1}H signals still observed after 20 min in ^{15}N HSQC-spectrum, filled circles observed after 32 h. The corresponding position of β-strands in the work by Quirk et al., 1999 is indicated by black bars above the secondary structure elements.

erence with appropriate conversion factors (Markley et al., 1998).

Extent of assignments and data deposition

With the exception of the His-tag and three residues in the unstructured N-terminus, all H^{N}, N and C^{α} resonances, and all but 6 C' and 6 H^{α} resonances were assigned. Assignments were deposited in the BioMagResBank (accession number BMRB-4329). The secondary structure shown in Figure 1 was based on chemical shift analysis and short range NOEs observed in ^{15}N NOESY-HSQC spectra and was supported by amide proton exchange data. Long-range H^{N}-H^{N} NOEs observed in a spectrum recorded on a 93% deuterated sample define a six-stranded parallel β-sheet with strand order 3, 2, 1, 4, 5 and 6. We note a discrepancy between our secondary structure model and the recently published model (Quirk et al., 1999) in the position of β-strands 3 and 4 in the sequence of transhydrogenase. This is unexpected considering the high sequence similarity between the domains.

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