Dissertation abstract

Energy-linked transhydrogenase is found in the cytoplasmic membrane of bacteria and in the inner membrane of mitochondria. It catalyzes the reversible hydride equivalent transfer between NAD(H) and NADP(H) coupled to proton translocation across the membrane. The enzyme has a tripartite arrangement of domains: the hydrophilic domains I and III harbor the binding sites for NAD(H) and NADP(H), respectively, and domain II contains the membrane-spanning region. In order to clarify the function of transhydrogenase, it is important to elucidate the conformationally driven mechanism by which the enzyme accomplishes the coupling between the redox-reaction and proton translocation.

The present study mainly focuses on structure-function relations of the hydrophilic domains of transhydrogenase from *Escherichia coli*. Both hydrophilic domains were cloned and expressed separately and found to form a catalytically active complex. The NADP(H)-binding domain III was shown to adopt different conformations dependent on the redox-state of the bound nucleotide. It was proposed that these structural differences play a central role in the conformationally-driven proton translocation mechanism of the intact enzyme.

An NMR characterization including NMR assignment and solution structures of domain III complexed with $NADP^+$ or NADPH was carried out. The structures showed that domain III essentially adopts a classical dinucleotide-binding fold composed of a twisted six-stranded parallell β-sheet flanked by helices on both sides. The NMR assignment was also used for chemical shift perturbation mapping in which the surface on domain III that is involved in interactions with domain I was identified. In addition, these experiments suggested that helixD/loop D (residues 391-411) and loop E (residues 427-433) in domain III exert redox-dependent regulations of the interface between domain I and domain III. Based on the NMR structure model, site-directed mutagenesis of residues involved in NADP(H) binding and/or interactions with domain I was carried out. It was concluded that loop E was particularly involved in regulating the release of NADPH, an event that is of central importance for the coupling mechanism in transhydrogenase.

Keywords: transhydrogenase, hydride transfer, proton translocation, NADP(H), NAD(H), dinucleotide-binding fold, NMR, site-directed mutagenesis.

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