DISSERTATION ABSTRACT

Proton-translocating transhydrogenase (TH) is a membrane-bound enzyme that couples hydride transfer between NAD(H) and NADP(H) to proton-pumping across the bacterial and mitochondrial inner membrane. The active dimer of transhydrogenase utilizes the electrochemical proton gradient to drive the reversible reduction of $NADP^+$ by NADH and translocate a proton to the cytoplasm and matrix, of bacteria and mitochondria, respectively. To elucidate the roles of different domains of *Escherichia coli* transhydrogenase in catalysis and assembly in the membrane, four constructs were made at a genomic level. In three constructs the α- and β-subunits of *E. coli* transhydrogenase were fused with 4, 16 or 26 amino acids resulting in a single polypeptide. In the fourth construct, the membrane domain of the β-subunit was split in two by the introduction of a stop codon, resulting in three polypeptides. Both split and the larger fused TH showed essentially wild type activities, whereas the two shorter fusion constructs catalyzed lower activities and showed an inability to correctly insert/assemble in the membrane. In split TH, a cross-link resulting from uniquely introduced cysteines constituted the first proof that helices 9 and 13/14, suggested to contribute to the proton channel are in close proximity of each other.

The mechanism of the redox-driven proton translocation by TH is suggested to involve long-range conformational changes. To obtain detailed information of what regions are important in proton translocation, NADP(H)-binding and substrate specificity, cysteine-scanning of helices 13 and 14, studies on the pH-dependence of the "hinge"-region and characterization of apo-domain III were performed. Helix 13 contains βAsn222, important for proton translocation, and four residues located on the same side of the helix gave inhibited activities when mutated. NADP(H)-binding was shown to be affected by residues mutated in helix 14 and the "hinge"-region, located in the β-linker. The latter is probably important for the long-range conformational coupling in the enzyme.

These results provided the basis for a hypothetical reaction mechanism of TH, as well as constructs possibly facilitating crystallization (fused TH) and low-resolution studies of the membrane domain by cross-linking (split TH).

Keywords: proton-translocating transhydrogenase, membrane protein, hydride transfer, NADP(H), NAD(H), cysteine-scanning, conformational coupling

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