DISSERTATION ABSTRACT

Proton-pumping nicotinamide nucleotide transhydrogenase (TH) from *E.coli* is an integral membrane protein found in most species. It is located in the inner membrane of mitochondria and in the plasma membrane of bacteria. TH catalyses the reversible transfer of hydride equivalents between NAD(H) and NADP(H), which is coupled to the translocation of protons across the membrane.

The aim of this thesis has been to study the relationship between the structure and the function of TH from *E. coli*. Using site-directed mutagenesis to introduce one or two cysteines in the cysteine-free TH followed by crosslinking, a packing-model of the transmembrane domain was proposed. It is suggested that, in the active tetramer, the 26 α helices are arranged in such a way that two separate proton channels are formed. To facilitate a more detailed study of this domain a TH was constructed containing a stop-codon introduced between two transmembrane helices. This functional split TH showed wild type like properties.

Characterisation of the double mutant D213C-R265C resulted in detection of a spontaneously formed disulfide bond, suggesting a close location of these two essential residues. A more thorough investigation of this pair led to the suggestion that Arg265 regulates the protonation/deprotonation of Asp213, which is likely to be in contact with the proton channel.

A 3D structure of the NADP(H)-binding domain III was predicted, and the properties of transmembrane helix 14 was characterised by cysteine scanning.

An investigation of isolated domain III revealed unspecific binding of NAD(H) to the NADP(H)-binding site. Based on this finding and the properties of some mutants of residues in the hinge-region, including Arg265, a pathway for the communication between the NADP(H)-binding site and the proton channel was suggested.

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

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