Abstract

Proton-translocating nicotinamide nucleotide transhydrogenase is an enzyme situated in the inner membrane of mitochondria and cytoplasmic membrane of bacteria that utilizes the electrochemical proton gradient to drive $NADP^+$ reduction by NADH. Transhydrogenase has been shown to be an important source of NADPH for biosynthesis as well as for detoxification of oxidative radicals generated during aerobic respiration. The enzyme has a tripartite organization; two soluble domains, I and III, containing the NAD(H)- and NADP(H)-binding sites, respectively, and an intervening membrane domain (domain II) containing the proton-translocation machinery.

The present study is concerned with the properties of the soluble domains I and III of *Escherichia coli* transhydrogenase. The structures of the individual domains as well as the complex between them were determined using a combination of NMR and X-ray crystallographic techniques. NAD(H) is bound in a cleft between the two dinucleotide-binding folds that comprise each monomer of the domain I dimer. Domain III binds NADP(H) at the C-terminal end of a beta sheet flanked by helices, constituting a classical dinucleotide-binding fold. The redox-sensitive loops D and E in domain III was shown by site-directed mutagenesis to be involved in both substrate binding and domain I-domain III interactions. Depending on the substrate redox state, domain III was shown to exhibit two different conformations in solution. The structure of the transient low affinity *E. coli* domain I-domain III complex was determined from the individual domain structures using paramagnetic relaxation enhancement-derived distance restraints as input in a rigid-body minimization.

It was serendipitously discovered that domain III displays substrate unspecificity in absence of NADP(H) both in the isolated domain III and in a number of mutants of the 'hinge'-region in the intact enzyme. We believe the observed unspecificity to be relevant for the mechanism of transhydrogenase, representing a transient state visited during the catalytic cycle of the enzyme in which substrate exchange occurs.

Keywords: transhydrogenase, NADP(H), NAD(H), hydride transfer, proton translocation, NMR, X-ray crystallography

ISBN 91-628-6809-8