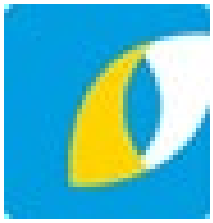

Abstract

The respiratory chains of the gram-negative bacterium *E. coli* have a flexible composition that enables survival in various environments from aerobic to anaerobic conditions. The aerobic respiratory chain of *E. coli* consists of several primary dehydrogenases and terminal reductases. Energy-converting NADH: ubiquinone oxidoreductase, also called respiratory complex I is the largest enzyme of the respiratory chains and serves as a primary dehydrogenase. It has an L-shaped form and couples the transfer of two electrons from NADH to ubiquinone (Q) with translocation of four protons across the membrane. Complex I consists of a peripheral and a membrane arm, it contains nine iron-sulfur clusters and one flavin mononucleotide (FMN) as cofactors. All cofactors for the electron transfer reaction are located in the peripheral arm, while proton translocation takes place in the membrane arm. The coupling of both processes is still under debate. The membrane arm contains three antiporter-like subunits NuoL, NuoM, NuoN, each comprising a putative proton pathway. A fourth proton pathway is contributed by other subunits building the so-called E-channel. It is not clear how the four protons are translocated across the membrane. It was proposed that either the four protons are translocated by four pathways or just to the pathway of the distal subunit NuoL.

Conformational changes play an important role in proton translocation. These movements need to be visualized to obtain a comprehensive understanding of a mechanism. Fourier-transform infrared (FTIR) spectroscopy is one of the most versatile techniques for the non-destructive characterization of proteins. Good-quality spectra are rapidly obtained with a high signal-to-noise ratio. However, detecting an intense absorption signal from a protein monolayer using FTIR spectroscopy is challenging. Surface-enhanced infrared absorption spectroscopy (SEIRAS) is applied to enhance the IR absorbance. An IR label is required to visualize conformational changes by means of IR spectroscopy. Nitrile labels are attractive IR labels as they can provide valuable information about the local environment of the probe in a defined protein environment. They appear in the clear region of IR spectra and do not overlap with protein signals. They are small and can be easily incorporated into a protein structure at distinct positions. Signals of nitrile labels

are sensitive towards hydrogen bond interactions making it possible to follow reaction-induced conformational changes.

To visualize a possible involvement of NuoM in proton translocation by complex I, a nitrile IR label was inserted in close proximity to its putative proton pathway. Individual residues were genetically changed to cysteine residues that were labelled with cyanides without blocking enzyme activity. Changes in the environment of the label were detected after addition of the substrates, NADH and Q (ubiquinone). The pattern of conformational changes at the distinct positions in NuoM suggests that NuoM is taking part in proton translocation.

Cytochrome *bd*-I oxidase belongs to terminal reductases of respiratory chains. They catalyze oxygen reduction to water. This process is coupled with ubiquinone oxidation to ubiquinol. *bd*-I oxidase consists of four subunits and contains three hems: b_{558}, b_{595} and heme *d* that are all located in one subunit. Conserved glutamic acid residues are in close proximity of heme b_{595} and heme *d*. Previous biochemical and spectroscopical studies revealed the importance of these positions, which show untypically high pK_a values. Here, these residues were mutated to aspartic acid and glutamine residues. The midpoint potential of the two hemes groups were drastically altered by the mutations. Unexpectedly, some of the mutants were still active. These findings are discussed in relation to the physiological role of the enzyme.