Effect of Different Substrates on Growth and Redox Potential Kinetics of Escherichia coli: Wild Type and Hydrogenases Lacking Mutant

Anna Poladyan, Satenik Mirzoyan, Armen Trchounian.

Department of Microbiology & Plants and Microbes Biotechnology and Research Institute of Biology, Yerevan State University, Yerevan, Armenia.

Escherichia coli is able to ferment different carbon sources and produce molecular hydrogen (H2) via four membrane-associated hydrogenase (Hyd) enzymes, as well as a respiratory chain with an alternative oxidase, an alternative external NADH dehydrogenase and a glycerol-phosphate dehydrogenase. (Cabrera-Orefice et al, 2014, Biochim Biophys Acta 1837(1): 73-84, 2014, Biochimie 102: 124-136. The alternative hydrogenases, seem to physiologically uncouple mitochondria in order to prevent ROS production (Guerrero-Castillo et al, 2011, Biochim Biophys Acta 1817(2): 353-62). In addition, the opening of an unspecific channel releases NAD+, thus inactivating complex I and decreasing the proton-pumping stoichiometry in the system. In regard to the carbon source, lactate or succinate led to the expression of AOX at all growth phases. In contrast, in the presence of glucose or galactose, AOX was only expressed at the late exponential phase. In contrast, in glycerol AOX was never expressed and biomass growth was larger. In addition, large lipidic production was observed. It is suggested that H. halnensis is useful to study cell adaptations to different growth conditions. Also its lipid production mechanisms might be interesting from a biotechnological point of view. The regulation of redox enzyme expression maintains a high respiratory activity even when ATP consumption is low, probably preventing overproduction of ROS.

Comparison of Dicyclohexylcarbodiimide (DCCD)-Induced Effects on Structure and Activity in Cytochrome c Oxidase (COX) from Bovine Heart and Rhodobacter Sphaeroides

Lawrence J. Prochaska, Kelli N. Fisher, Christine N. Pokalsky.

Biochemistry and Molecular Biology, Wright State University, Dayton, OH, USA.

Previous work has shown that DCCD inhibits bovine heart cytochrome c oxidase (COX) function by binding to Glu-90 in subunit III (SIII). The goal of this study was to compare the effects of DCCD modification on structure and electron transfer in COX from bovine heart and Rhodobacter sphaeroides. DCCD modification induced similar shifts in migration of SIII on SDS-PAGE focusing on the ratio of native COX to COX with SIII modification. DCCD modification dependence for the inhibition of electron transfer by DCCD was also similar in both enzymes. The pH dependence of electron transfer activity in the DCCD-modified enzymes was acid shifted 0.7-0.9 pH unit in both subunits. However, bovine heart COX treated with DCCD exhibited less inhibition of electron transfer activity at pH 9.5-10.0 than at lower pH, while DCCD treated R. sphaeroides COX had less inhibition at pH 6.5-7.0 than at higher pH. The heme circular dichroism spectrum showed that DCCD induced a red shift of 0.5-0.8 nm in both enzymes when monitored at both pH 7.0 and 10.0. Steady-state heme a reduction during electron transfer increased from 18.8% to 33±2% in DCCD-modified beef COX at pH 7.0, while in R. sphaeroides, it remained unchanged from 41±2% to 36±2%. In summary, our results indicate that DCCD, while binding at a similar site in SIII, leads to different effects in the two enzymes. DCCD modification of SIII of bovine heart may cause blockage of the putative O2 transfer pathway, while in R. sphaeroides it may induce a slowed proton uptake. The different characteristics of inhibition may be due to variation in both the number of subunits and their structural homology between the two forms of COX.

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico, Mexico.

Debaryomyces hansenii is an oleaginous, mostly aerobic yeast. Its mitochondrial respiratory chain contains complexes I, II, III and IV, an alternative oxidase, an alternative external NADH dehydrogenase and a glycerol-phosphate dehydrogenase. (Cabrera-Orefice et al, 2014, Biochim Biophys Acta 1837(1): 73-84, 2014, Biochimie 102: 124-136. Alternative hydrogenases, seem to physiologically uncouple mitochondria in order to prevent ROS production (Guerrero-Castillo et al, 2011, Biochim Biophys Acta 1817(2): 353-62). In addition, the opening of a unspecific channel releases NAD+, thus inactivating complex I and decreasing the proton-pumping stoichiometry in the system. In regard to the carbon source, lactate or succinate led to the expression of AOX at all growth phases. In contrast, in the presence of glucose or galactose, AOX was only expressed at the late exponential phase. In contrast, in glycerol AOX was never expressed and biomass growth was larger. In addition, large lipidic production was observed. It is suggested that H. halnensis is useful to study cell adaptations to different growth conditions. Also its lipid production mechanisms might be interesting from a biotechnological point of view. The regulation of redox enzyme expression maintains a high respiratory activity even when ATP consumption is low, probably preventing overproduction of ROS.

Regulation of the Reaction between Cytochrome c and Cytochrome Oxidase

Jennifer Silva-Nash, Francis Millett, Martha Scharlau.

Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, USA.

Irreversible brain damage is seen in patients that have suffered strokes, cardiac arrest, or other brain ischemia events. The hypoxic conditions result in neuron death, and studies have shown that additional damage occurs when blood flow is restored. It is thought that the lack of energy production during post-ischemia events also causes brain damage, as the brain depends on oxidative phosphorylation. Cytochrome c (Cyt c) plays a crucial role in energy production by means of the electron transport chain, transferring electrons between complexes III and IV (cytochrome c oxidase, CoO). Mitochondrial release of Cyt c into cytosol results in type II apoptosis, and release within the mitochondria suggests that Cyt c detoxifies reactive oxygen species (ROS). Reversible phosphorylations at tyrosine (Y) residues of Cyt c and CoO have proven to alter electron transfer kinetics and allosteric regulation. Specifically, brain ischemia events result in dephosphorylation, and signals promoting cell growth result in phosphorylation. We sought to determine whether the phosphorylation of a Y residue, Y97, affects binding kinetics between Cyt c and CoO, regulating the role of Cyt c. We hypothesized that comparison of binding kinetics of mutated Y97E Cyt c and wild-type Cyt c to CoO, in an in vitro study would reveal effects of phosphorylation at this residue. Purification of phosphorylated Cyt c has proven to be a difficult process by traditional methods, but exchanging residues can mimic the effect of phosphorylation. Y97 was replaced by glutamic acid in the negative charge resembling the phosphate group in phosphorylated Cyt c. Both mutant, Y97E, and wild-type, Y97, were synthesized and purified, then subjected to analytical ultracentrifuge and laser photolysis to examine binding kinetics. Results from analytical ultracentrifuge and laser photolysis revealed little difference in binding between mutant and wild-type Cyt c.

Genetic Regulatory Systems

The Computational Determination of Small RNA Binding Constant to Clarify the Synthetic Regulatory Circuit in Escherichia coli

Cheng-Ping Jheng1, Shih-Wei Wang2, Kuan-Ling Chen1, Tsu-Han Chen1, Shyng-You Chou2, Wan-Sheng Su3, Po-Han Lee4, Cheng-I Lee2.

1Life Science, National Chung Cheng University, Taiwan, Chia-Yi, Taiwan, 2Stevenson School, Pebble Beach, CA, USA, 3Electrical and Computer Engineering, Cornell University, New York, NY, USA, 4Medical Applied Chemistry, Chung Shan Medical University, Taichung, Taiwan, 5Affiliated Senior High School, National Taiwan Normal University, Taipei, Taiwan, 6National Applied Research Laboratories, National Center for High-Performance Computing, Hsinchu, Taiwan, 7Life Science, National Chung Cheng University, Chia-Yi, Taiwan.

Small regulatory RNA (sRNA) is responsible for coordination of gene expression network in both prokaryotes and eukaryotes. Some critical genetic regulation circuits in prokaryotes is mostly controlled by the binding of sRNA and the related target mRNA. In this study, we analyze the phase space of the synthetic regulatory circuit in Escherichia coli by utilizing molecular dynamic simulations to calculate their related binding constants. The computed results