1544-Pos Board B521

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

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Escherichia coli is able to ferment different carbon sources and produce molecular hydrogen (H2) via four membrane-associated hydrogenase (Hyd) enzymes. The role of oxidation reduction potential (ORP) for bacterial metabolism and regulation of Hyd enzymes is stated. Moreover, relation of Hyds to F0F1-ATPase was also established during bacterial carbon source fermentation.

The growth, ORP kinetics, proton-potassium exchange and the FoF1-ATPase activity of *E. coli* wild type BW25113 and Hyds lucking ΔhypF mutant were investigated upon different carbon sources utilization at pH 6.5 and 7.5. The essential role of Hyd enzymes for bacterial growth was shown upon glucose fermentation at both pHs: in mutant cells up to 2 times growth suppression was observed compared with wild type. Wild type ORP drop up to −400 −600 mV was observed upon glycerol, formate or glucose fermentation. Enhanced H2 production and 1.1- to 1.3-fold stimulation of bacterial growth were observed upon substrates co-supplementation. Whereas, during mutant growth ORP decrease only to −150 to 220 mV was observed upon glycerol or glucose fermentation at both pHs. Hyds participation in H^+ extrusion was shown, but during glycerol fermentation their input is more than in glucose fermenting cells; moreover, during glucose fermentation depending of medium pH they have different relationship and role in potassium ions uptake and transport systems operation. During mixed carbon fermentation (glucose and glycerol) for the FoF1-ATPase activity of E. coli alkaline pH is more optimal. ATPase activity in ΔhypF mutant was suppressed at pH 7.5 which shows some interaction between Hyds components with FoF1.

The results point out the significant role of substrates alone or their mixed combination and Hyd enzymes for *E. coli* growth, ORP formation and H2 production.

1545-Pos Board B522

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

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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 in both enzymes, suggesting similar SIII modification. The concentration 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 enzymes. 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 ± 1% 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 differential 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 forms.

1546-Pos Board B523

Debaryomyces Hansenii: Adaptation Mechanisms to Different Carbon Sources and Oxygen Concentrations

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Debaryomyces hansenii is an oleaginous, mostly aerobic yeast. Its mitochondrial respiratory chain contains complejxes I, II, III and IV, an alternative oxidase, aln alternative external NADH dehydrogenase an a glycerol-phosphate dehydrogenase (Cabrera-Orefice et al, 2014, Biochim Biophys Acta 1837(1): 73-84; 2014, Biochimie 102: 124-136). Alternative dehydrogenases, 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 D. hansenii 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.

1547-Pos Board B524

Regulation of the Reaction between Cytochrome c and Cytochrome Oxidase

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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, CcO). 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 CcO 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 CcO, regulating the role of Cyt c. We hypothesized that comparison of binding kinetics of mutated Y97E Cyt c and wild-type Cyt c, to CcO, 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 glutamate (E)− 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

1548-Pos Board B525

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

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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 presentation, 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