its lack leads to an incomplete repression of mitochondrial respiration in presence of glucose [1]. Voltage Dependent Anion Channel 1 (VDAC1) is the protein that ensures the exchanges of metabolites and ions through the mitochondrial outer membrane (MOM). Saccharomyces cerevisiae cell lacking VDAC1 (Δpor1) show a depressive growth on not fermentable carbon source. In a recent work, we demonstrated that the expression of human SOD1 in Δpor1 restores the yeast growth on glycerol, the mitochondrial functionality and the ROS level [2]. Moreover the presence of hSOD1 increases the gene expression of β-barrel MOM proteins. Among them, the second VDAC isoform (also known as porin2) was found 8 times over-expressed [2]. The pore-forming activity of yeast VDAC2 has never been characterized. Our results strongly suggest that it can substitute for VDAC1 function. Accordingly, we observed that the expression of SOD1 in a yeast strain devoid of both VDAC1 and VDAC2 is unable to reactivate the mitochondrial metabolism. In this work we present these observations and a preliminary characterization of purified S. cerevisiae VDAC2.

References


07.11

Beneficial and deleterious roles of mitochondrial ROS upon inflammation

Johanna-Cathartina Duvigneau1, Andrea Müllebnerah, András Meszaros1, Adelheid Weidingera, Gabriel Dorighellob, Sergiu Dumitrescu1, Heinz Redlb, Mihaly Borosc, Andrey V. Kozlovb.

1Institute of Medical Biochemistry, University of Veterinary Medicine, Vienna, Austria
2Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Center, Vienna, Austria
3Institute of Surgical Research, University of Szeged, Hungary

E-mail address: Andrey.kozlov@trauma.lbg.ac.at (A.V. Kozlov)

The aim of this study was to clarify the impact of mitochondrial reactive oxygen species (mtROS) upon inflammation induced by bacterial toxin in rats and in rat hepatocyte and macrophage cultures incubated with inflammatory mediators (IM). Inflammation influenced mtROS generation in two manners, namely elevating mtROS generation within mitochondria and releasing mtROS into cytoplasm, tentatively via mPTP. The increase in mtROS levels inside and outside of mitochondria caused oxidative stress and activated specific signaling pathways. The oxidative stress was manifested as a drop in mitochondrial membrane potential and a damage to cellular membrane resulted in the release of intracellular enzymes (AST, LDH) in the blood. The increase in mtROS resulted in up-regulation of IM (iNOS and IL-6) accompanied by a drastic increase in intracellular and blood NO levels; in addition it resulted in an increase in NADPH-oxidase activity in macrophages. The treatment with mitoTEMPO, a mitochondria targeted antioxidant, decayed mtROS, normalized membrane potential, decreased expressions of iNOS and IL-6 in hepatocytes, decreased NADPH-oxidase activity in macrophages and reduced circulating NO and AST/ALT levels in the blood. In addition we demonstrated that the increase in mtROS was induced by late phase IM such as IL-4 released during compensatory anti-inflammatory response phase rather than by IM such as TNF-alpha released during systemic inflammatory response, an early acute phase. The nature of the IM is still unclear. All together our data suggest that mtROS predominantly affect inflammatory response not via oxidative stress reactions but via two signaling pathways, namely up-regulation of inflammatory genes and elevation NADPH-oxidase activity. Since both these pathways are components of the immune response, the application of mitochondria targeted antioxidants can be both deleterious weakening immune system and beneficial attenuating host tissue damage.

doi:10.1016/j.bbabio.2016.04.177

07.12

Structural insights into biological hydrazine synthesis

Christina Ferousia, Andreas Dietla, Jutta J. Maaclkeb, Frauke Baymannb, Jan T. Keltjenc, Mike S.M. Jettenb, Thomas R.M. Barendsb, Boran Kartald, Joachim Reimmanb.

aDepartment of Microbiology, Radboud University, Heyendaalseweg 135, 6525AJ Nijmegen, the Netherlands
bDepartment of Biomolecular Mechanisms, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany

Anaerobic ammonium oxidizing (anammox) bacteria are ubiquitously present in nature and contribute substantially to the global release of fixed nitrogen to the atmosphere. The biological novelty of the anammox process is the production of the reactive intermediate hydrazine (N₂H₄), which gets subsequently oxidized to dinitrogen gas (N₂) yielding the necessary reducing equivalents for respiration. The protein complex catalyzing the formation of N₂H₄ from nitric oxide (NO) and ammonium (NH₄⁺), that is Hydrazine Synthase (HZS), was purified to homogeneity as a dimer of heterotrimers (α₂γ₂). Analyses of the crystal structure of HZS at 2.7 Å resolution together with spectroscopic studies led to the formulation of a working hypothesis. We suggest that hydrazine synthesis occurs via a two-step mechanism facilitated by two heme c active sites. First, NO undergoes a three-electron reduction to hydroxylamine (NH₂OH) at the active site of the γ-subunit (heme yl). NH₂OH is then transferred to the α-subunit through an intraprotein tunneling system that is presumably regulated by a short amino acid stretch of the β-subunit. The second active site heme of the HZS complex (heme αd) facilitates the condensation of ammonia (NH₃) and NH₂OH to the end product N₂H₄. Interestingly, the resolution of the structure of HZS revealed few oddities with regards to the architecture of the active site hemes. The γ-subunit is structurally similar to cytochrome c peroxidase and methylene utilization protein G but displays a third covalent attachment between a cysteine residue and the porphyrin ring of the yl heme moiety. Heme αd also differs considerably from a canonical heme c site, as the histidine residue of the CXXCH heme binding motif does not coordinate the heme iron, but a zinc ion instead. The fifth ligand of heme αd appears to be a tyrosine, resembling the active site of catalases. These results provide the first insight into the molecular mechanism of biological hydrazine synthesis.


07.13

A new answer to an old problem: The energization of brain mitochondria with pyruvate is regulated by cytosolic calcium via the mitochondrial gas pedal and does not require the mitochondrial Ca uptake via the Ca uniporter- New evidences from experiments with MCU− mice

Frank N. Gellerichab, Nikki Karavassilia, Rodrigo Herrera-Molinaab, Stefan Vielhabera, Wolfram S. Kunze
Department of Epileptology and Life and Brain Centre, University of Bonn, Germany
E-mail address: c.ferousi@science.ru.nl (C. Ferousi)

It is assumed that for activation of mitochondria, cytosolic Ca²⁺ has to enter the matrix via the Ca uniporter (MCU) and to stimulate the matrix dehydrogenases. However, recent experiments from Pan et al., show that animals without MCU can live well although MCU⁻ mice cannot acutely take up Ca²⁺. Obviously the classic hypothesis is insufficient to explain these results. Recently, our group discovered that increasing Ca²⁺ (R0.5 = 300 nM) can activate via aralar (glutamate/aspartate carrier) the activity of both the malate aspartate shuttle (MAS) and the formation of pyruvate without involvement of MCU. As result pyruvate and hydrid anions are increasingly pushed into mitochondria. We called this process mitochondrial gas pedal. To prove our hypothesis, we performed experiments with MCU ko and WT mice. By means of Morris water maze test we found that the MCU knock out did not affect hippocampal memory function and the swimming performance as well. Isolated brain MCU⁻ mitochondria did not accumulate Ca²⁺ and were stable against large Ca²⁺ additions. Glutamate/malate but not pyruvate/malate driven respiration was at least doubled by increasing Ca²⁺ in WT and ko mitochondria with and without ruthenium red. Respiration of glucose fed synaptosomes was decreased by BAPTA due to diminished Ca²⁺. Pyruvate addition partially recovered the respiration indicating the contribution of limited pyruvate to the inhibition of respiration. In contrast the inhibition of respiration by aminooxyacetate not affecting Ca²⁺ could be completely reversed by pyruvate addition. We obtained similar results with MCU⁻ and WT synaptosomes indicating, that the MCU is not involved in the Ca²⁺-regulation of pyruvate oxidation of brain mitochondria. In brain mitochondria the extent of the gas pedal mechanism is larger than the classic intramitochondrial Ca²⁺-activation of matrix DH. This is the reason that mitochondrial energization can also be regulated by cytosolic Ca²⁺ in MCU ko animals.

doi:10.1016/j.bbabio.2016.04.179

07.14

Respiratory complex II catalyzed ROS production

Vera G. Grivennikova, Andrei D. Vinogradov
Department of Biochemistry, School of Biology, Moscow State University, Moscow, Russian Federation
E-mail address: adv@biochem.bio.msu.su (A.D. Vinogradov)

Complex II plus complex III contribute about 20% to the overall ROS production by the mammalian respiratory chain components [1]. A bell-shaped dependence of ROS-producing complex II activity on succinate concentration have been described [2,3]. Here we present further analysis of complex II ROS generating activity. The succinate-reduced membrane-bound respiratory complex II in inside-out bovine heart submitochondrial particles freed of superoxide dismutase and treated with rotenone and myxothiazol reacts with oxygen (at 50 μM “optimal” succinate concentration) at the specific rate of about 5% of its NADH-supported fumarate reductase activity. The enzyme produces more than 80% of ROS as superoxide anion. The reaction is insensitive to Arpenin S (the inhibitor of iron–sulfur S3–ubiquinone junction site) and its rate decreases upon increase of succinate concentration. The contribution of complex II to the ROS production by myxothiazol-inhibited, NADH-reduced respiratory chain (complexes I + II) is about 15%. The dependence of ROS production on succinate/fumarate ratio shows that the midpoint redox potential of oxygen-reactive component is about 40 mV more positive than that of succinate/fumarate couple, a value close to that of S3 iron–sulfur center. A possibility of the dicarboxylate binding-induced conformational change of the enzyme structure propagated to S3 specific protein environment is proposed.

References
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