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S17 System Bioenergetics

17L1

Antioxidant effects induced in biological macromolecular systems by high density photons through localized excitations

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We have recently reported that high density photon fluxes (HDGP), which induce multiphoton processes, generate antioxidant effects in biological systems subjected to ROS (reactive oxygen species) aggression (J.Photochem.Photobiol.B-102, 39–44, 2011). In this paper we present experimental results obtained on protein macromolecules subjected to ultraviolet denaturation. Irradiation with HDGP, in the visible band ($\lambda = 515$ nm), generates localized excitations by polarization effects, with strong antioxidant properties. We studied two specific proteins: superoxide dismutase (SOD), a relevant free radicals "scavenger", and bovine serum albumin (BSA). Under ultraviolet (UV) denaturation, the SOD-enzyme, when simultaneously irradiated with $\lambda = 515$ nm light, preserves its entire activity through a photo isomerization of the Cu–Zn link at the enzymic active center. The UV-irradiation induces a breaking of BSA-molecule as revealed in circular dichroism spectroscopy, with a significant loss of its α-helix content. In our experimental set-up, when BSA is previously irradiated with HDGP, the induced localized excitations reduce the loss of α-helix content from 2.25% to 1.65%. Circular dichroism spectra performed on cellular cultures under UV-irradiation, reveal, when simultaneously irradiated with green light, a protection of the proteins helicticity, through photo isomerization of l–d histidine in the secondary amino acids chain structure. A quantum chemical computation was used to investigate the theoretical basis for these oxidation processes in order to calculate the energetic structure of these molecular states. The calculations were performed using a Gaussian 03W with the Chemisian software. We term biological spectroscopy, this new type of research, in which biological systems are used as detector of subtle physical transitions.

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17L2

Dehydrogenase kinetic parameters determine the electron competition mechanisms in respiratory chain

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In *Saccharomyces cerevisiae*, the most important systems for conveying excess cytosolic NADH to the mitochondrial respiratory chain are the external NADH dehydrogenases (Nde1p and Nde2p) and the glycerol-3-phosphate dehydrogenase shuttle. In the latter system, NADH is oxidized to NAD^+ and dihydroxyacetone phosphate is reduced to glycerol-3-phosphate (G3P) by the cytosolic Gpd1p. G3P donates electrons to the respiratory chain via mitochondrial G3P-dehydrogenase (Gut2p). At saturating concentrations of NADH, the activation of external NADH dehydrogenases completely inhibits glycerol 3-phosphate oxidation. This inhibition is caused by competition for the entrance of electrons into the respiratory chain. Using single deletion mutants of Nde1p or Nde2p, we have shown that glycerol-3-phosphate oxidation via Gut2p is inhibited fully when NADH is oxidized via Nde1p, whereas only 50% of glycerol-3-phosphate oxidation is inhibited when Nde2p is functioning. Moreover, we show that electrons from Nde1p are favored over electrons coming from Ndip (internal NADH dehydrogenase) and that when electrons come from either Nde1p or Nde2p and succinodehydrogenase, their use by the respiratory chain is shared to a comparable extent [1]. This suggests a specific competition for electron entrance into the respiratory chain, which is not due to the supramolecular organization of the respiratory chain [2]. Such a competition generates a priority for cytosolic NADH reoxidation. By determining the kinetic parameters of different external dehydrogenases and using a stochastic model derived for the complex I, we showed that the different processes of electron competition observed in various yeast strains may be due to the particular kinetic parameters of the involved dehydrogenases.

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17L3

Conversion of *Corynebacterium glutamicum* from an aerobic respiring to an aerobic fermenting bacterium by inactivation of the respiratory chain

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0005-2728/$ – see front matter.

Corynebacterium glutamicum is a member of the gram-positive bacteria with high G + C content and a model organism in industrial biotechnology [1,2]. Its respiratory chain comprises a cytochrome bc_1–aa_3 supercomplex [3] and a cytochrome bd oxidase [4]. Oxidative phosphorylation is not essential as a strain lacking F_1F_O-ATP synthase was viable and showed reasonable growth [5]. In this study three respiratory mutants were characterized which lacked either the bd branch (ΔcydAB), or the bc_1–aa_3 branch (Δqcr), or both. Lack of bd oxidase was inhibitory only under conditions of oxygen limitation, whereas the absence of a functional bc_1–aa_3 supercomplex led to decreases in growth rate, biomass yield, respiration and proton motive force (pmf) under oxygen excess. These results show that the bc_1–aa_3 supercomplex is of major importance for aerobic respiration. For the first time, a *C. glutamicum* strain with a completely inactivated aerobic respiratory chain was obtained (ΔcydABΔqcr), named DOOR (devoid of oxygen respiration). In glucose minimal medium supplemented with peptone, DOOR showed growth based on a fermentative type of catabolism with l-lactate as major and acetate and succinate as minor products. No decrease of the dissolved oxygen concentration could be detected for the DOOR mutant, indicating the absence of alternative terminal oxidases. The proton motive force of the DOOR mutant was reduced by about 30%. Candidates for pmf generation in this strain are succinate:menaquinone oxidoreductase and F_1F_0-ATP synthase.

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17L4

Combining quantitative live-cell microscopy with mathematical modeling to understand and combat mitochondrial disease in skeletal muscle

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Mitochondria are at the heart of cellular bioenergetics with many biochemical pathways conversing in the mitochondria. The main catabolic pathway is ATP production by the oxidation phosphorylation (OXPHOS) system. Mitochondrial diseases are often the consequence of a genetic defect in one or more complexes of OXPHOS. In particular NADH–ubiquinone oxidoreductase, also known as complex I, is often affected, as it comprises 45 subunits encoded by both the nuclear and mitochondrial genome. As of now, no cure exists for mitochondrial diseases [1,2].

It is important to understand cellular metabolism in OXPHOS dysfunctional cells in order to initiate pharmacological interventions in mitochondrial disease cases. To achieve this goal, we use a systems biology approach of mathematical modeling, classic "omics" approaches and live-cell imaging to develop predictive mathematical models of cellular bioenergetics in healthy skeletal muscle and in diseased skeletal muscle of mice harboring a defective complex I ($NDUFS4^{-/-}$ knock-out) [3].

In order to study cellular and mitochondrial metabolism in cultured myoblasts and myotubes, we apply quantitative live-cell microscopy to measure metabolites by fluorescent FRET-based biosensors. This allows us to visualize and study *in situ* specific changes that would be missed by cell population based "omics" techniques. To understand both anabolic and metabolic processes, we currently focus on glucose and ATP dynamics in living cells [4]. Here we will report on our approach of combining mathematical modeling and quantitative live-cell imaging. Additionally, fluorescent biosensors are new tools that will help us to further understand cellular and mitochondrial bioenergetics.

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17P1

Escherichia coli membrane vesicles F_0F_1-ATPase activity under glycerol fermentation at alkaline and acidic pH

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Escherichia coli is able to ferment glycerol and produce molecular hydrogen (H_2) by using different hydrogenases (Hyd) [1,2]. The most important aspect in regulation of H_2 production by *E. coli* formate hydrogen lyase (FHL) and its energetics is the requirement of the F_0F_1-ATPase: a relationship between these hydrogenases and F_0F_1 was assumed [2,3]. In *E. coli*, FHL is a complex that consists of two enzymes, formate dehydrogenase H (Fdh-H) and Hyd. It has been shown that Hyd-2 is mostly responsible and Hyd-1 less responsible for H_2 production at alkaline pH [3]. The latter was sensitive to *N*,*N*′-dicyclohexylcarbodiimide (DCCD), inhibitor of the H^+-translocating F_0F_1-ATPase.

In this study we investigated overall and DCCD-sensitive ATPase activity of glycerol-fermented *E. coli* wild type BW25113, *E. coli selC* (with deficiency of Fdh-H, Fdh-O, Fdh-N), and *E. coli selC hyaB hybC* (with Fdh-H, Fdh-O, Fdh-N, HyaB, HybC deficiency) mutant strains membrane vesicles at different pHs.

ATPase activity of membrane vesicles was higher at pH 7.5 in wild type BW25113 compared with that at pH 5.5 (~4-fold; $p \leq 0.05$). DCCD inhibited markedly ATPase activity ~11-fold ($p \leq 0.05$) in wild type at pH 7.5, but at pH 5.5 — ~1.5-fold ($p \leq 0.025$). Compared with wild type cells, ATPase activity at pH 7.5 was lowered in ~1.5-fold