Oxidative Phosphorylation & Mitochondrial Metabolism

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Crystallographic and Functional Evidence for Involvement of the Flexible C-Terminus of Subunit I of Rhodobacter Sphaeroides Cytochrome C Oxidase in Proton Pumping

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The 4-subunit crystal structure (PDB:1M56) of Rhodobacter sphaeroides cytochrome c oxidase (RsCcO) does not resolve the last six residues of the C-terminus of subunit I. In the higher resolution 2-subunit structure (PDB: 2GSM), ten more residues in the C-terminus are missing, suggesting flexibility of this region, which is also implied by deuterium exchange and computational analysis. To determine the functional significance of the C-terminus, three mutants of RsCcO were created, CIΔ16 , CIΔ6 and E552A. The 16-residue deletion mutant, CIΔ16 (C-terminus = T550), showed suicide inactivation and lower steady state activity compared to wild type RsCcO. In the purified, reconstituted CIΔ16, proton pumping is greatly impaired, though its respiratory control is good. SDS-PAGE indicates that subunit III is partially lost. The shorter deletion mutant, CIΔ6 (C-terminus = W560), had normal activity and no suicide inactivation, suggesting that loss of activity in CIΔ16 could be due to the importance of some residue(s) in the 551-560 region. A conserved glutamate, E552, was a likely candidate, and in fact the E552A mutant had normal activity but diminished proton-pumping. Crystal structures of CIΔ16 and CIΔ6 were obtained at 2.1 Å and 2.5 Å respectively. The CIΔ16 structure shows a rearrangement of the new C-terminus (T550) to interact directly with H26, but the ordered waters above D132 in the D-pathway were unaltered. The CIΔ6 structure appeared WT. A role for E552 in proton pumping is implied, and of the 551 to 560 region in stabilizing the association of subunit I and III. (NIH GM26916).

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The Subunit B-Dimer of the F1-ATPase Influences Conformational Transitions During Catalysis

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The F1FO-ATP synthase, the enzyme responsible for most of ATP generated in biological systems, is a highly asymmetric enzyme complex. Part of the asymmetry stems from interactions of the nucleotide binding subunits α and β with the central rotor subunit gamma. Subunit gamma transfers the energy of the proton-driven rotation of the FO subunit c ring to subunit β, and induces conformational changes that drive ATP release during synthesis. We hypothesize that interactions with the external stalk subunit b-dimer will introduce additional asymmetry to the enzyme complex that may influence catalytic transitions. To investigate our hypothesis, we substituted two tyrosines in subunit β at amino acid positions 331 and 354 for cysteines in an otherwise cysteine-less enzyme. These tyrosines were shown previously to be part of the catalytic and non-catalytic sites, respectively. Using the cysteine-less enzyme as a control, our experiments showed that trapping efficiency of the cysteine-less protein as well as both mutants using AlFx- in what is considered to be a transition-state like conformation was influenced by the presence of soluble b2. Reactivation of trapped enzyme occurred in presence of excess of MgATP and was also influenced by the presence of soluble b2.

In separate experiments using site-specific spin labeling and two different spin labels as reporter groups, we observed changes in the conformation of both the catalytic and non-catalytic sites during trapping. In each case, the presence of subunit b-dimer resulted in changes in the conformational transitions. Experiments are underway to localize the binding site of the subunit b-dimer in either the catalytic or the non-catalytic cleft of the ATPase.

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The C-Subunit Ring of the F1FO ATP Synthase Constitutes a Leak Channel that Regulates Cellular Metabolic Efficiency by Counteracting the H+ Translocator

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The F1FO ATP synthase is a multiple protein mitochondrial inner membrane complex that is responsible for the production of energy in the form of ATP for eukaryotic cells. It is known that the efficiency of ATP production by the F1FO ATP synthase is attenuated by an inner mitochondrial membrane leak of hydrogen ions. Previous work has shown that this leak is regulated and that its modulation protects neurons from cell death stimuli. Nevertheless, the exact molecular identity of the proton leak channel is unknown. Herein we describe, using electrophysiological techniques, a previously undetected non-selective ion channel in the c-subunit ring of the ATP synthase. The channel conductance is markedly decreased by adenine nucleotides and blocked by recombinant beta-subunit of the synthase. Mutation of highly conserved glycine residues within the putative channel pore increases channel conductance, attenuates responses to ATP, and compromises cell function leading to cell death. We conclude that the c-subunit ring is a highly regulated ion channel that can leak protons and other cations in order to regulate the degree of metabolic efficiency. The channel activity described in this work is different from the known characteristics of H+ tranlocator. We predict that normal c-subunit leak channel activity may become compromised during pathological events, disrupting inner membrane integrity.

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Bioenergetics of Contractile Function in Heart Trabeculae from Diabetic Rats

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Morbidity in non-insulin dependent diabetes mellitus (NIDDM) patients is commonly linked to cardiomyopathy, but the mechanisms involved in heart dysfunction are not well understood. In this study, the Zucker diabetic fatty (ZDF) rat was utilized as an animal model of NIDDM. Herein we present a comparative bioenergetic characterization of working heart trabeculae from ZDF and its lean control subjected to changes in workload through pacing frequency. We monitored in parallel the developed force and respiratory rate in the absence and presence of increasing levels of insulin. In the absence of insulin, and compared to lean controls, trabeculae from ZDF rats showed a decreased dependence of the respiratory rate as a function of pacing frequency. Respiration was stimulated by increase pacing frequency in the presence of 10 nM Insulin in the lean control but not in the ZDF-derived trabeculae. In contrast, at 100 nM Insulin, respiratory fluxes in both ZDF- and lean control-trabeculae were rather insensitive to the frequency changes.

The relatively lower respiratory rates displayed by ZDF with respect to lean heart trabeculae is in agreement with respiration measurements performed in mitochondria isolated from these hearts. In the presence of substrates of complex I, II and IV, ZDF mitochondria displayed 30% to 50% reduction in respiratory state 3 and uncoupled activities, together with decreased NADH and mitochondrial membrane potential (DYm). Additionally, the build-up of DYm as a function of glutamate/malate concentration required higher levels of substrate in heart mitochondria from obese as compared to lean rats.

Taken together, these results indicate that a compromised mitochondrial function may be at the origin of the reduced contractility exhibited by heart trabeculae from the obese ZDF rat.

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Palmitate Improves Redox Balance and Enhances Contractility While Offsetting Adverse Effects of Hyperglycemia in the Diabetic Cardiomyocyte

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High blood glucose and fatty acid levels constitute main hallmarks of diabetes. In heart from diabetic animals, wide variations in plasmatic glucose combined with sympathetic hyperactivation represent a challenge to mitochondria that have to supply energy and maintain redox balance. Our previous data in db/db mice unveiled a significant redox imbalance in heart cells under combined hyperglycemia (HG) and beta adrenergic stimulation (via isoproterenol, ISO), leading to cardiomyocyte dysfunction. Herein, we address the role of palmitate (Palm) upon the adverse effects of HG+ISO on diabetic cardiomyocyte contractility and intracellular redox status. The ISO (10nM) response of WT vs db/db myocytes subjected to 5mM and 30mM glucose, in the absence and presence of 0.4mM (WT) and 0.8mM (db/db) Palm, was examined comparatively with respect to: i) fractional sarcomere shortening (FS) and Ca2+ transients, and ii) the intracellular status of NADH, reduced glutathione (GSH) and reactive oxygen species (ROS). Both WT and db/db myocytes exhibited a significant increase in ISO response with Palm under 5mM glucose: FS increased by 320% and 323% in WT and db/db, $p<0.01$ vs 193% and 178%, respectively, no Palm. Under HG, FS was similarly enhanced in WT with Palm (372% vs 181% with no Palm, $p<0.01$). Diabetic myocytes showed lower contractility in HG+ISO (94%, $p<0.01$ vs WT) with Palm enhancing the ISO response to 380%. Under every condition, Palm strongly decreased ROS (15- to 60-fold) and significantly increased GSH (20% to 40%) in both WT and db/db. NADH was significantly increased in WT (40% to 50%) but decreased with db/db (30% to 35%). These findings reveal that Palm enhances contractility of db/db myocytes via reduction of the intracellular redox environment, thus overcoming the adverse effects of HG under ISO stimulation.