S9.L3

Regulation of mitochondrial cytochrome c oxidase by calcium and sodium ions via special cation binding site

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Cytochrome c oxidase (CcO) from bovine heart contains a special Cation Binding Site (CBS) located in subunit I near the outer face of the mitochondrial membrane close to heme a. The site binds divalent ($Ca^{2+} \gg Mg^{2+}$) and monovalent cations ($Na^+ > Li^+ > K^+$). Divalent cations shift the absorption spectrum of heme a, which allowed previously to determine the kinetics and equilibrium characteristics of Ca^{2+} binding at the CBS, but functional significance of the cation binding remained unknown until recently. As found in this group, extramitochondrial Ca^{2+} inhibits 2–3-fold activity of the mitochondrial or isolated bovine CcO with Ki of ~1 μM, close to Kd of Ca^{2+} binding [1]. Interestingly, the inhibition is observed only at low, turnover rates of the enzyme (~10 s^{-1} or less) and is fully lifted under the conditions of conventional CcO activity assays at TN close to Vmax, which explains why the effect of Ca^{2+} was not noticed earlier. Na^+ ions that compete with Ca^{2+} for the CBS, do not affect themselves with the activity but protect CcO from the inhibitory effect of Ca^{2+}. At physiological concentrations of Na^+ in the cytoplasm (5–10 mM), the Kd for Ca^{2+} binding with CcO raises from ~1 μM to ~10 μM. Accordingly, CcO is likely to be protected from inhibition by Ca^{2+} in the resting cells, but the inhibition may come into play during the peaks of Ca^{2+} release at the mitochondria/ER interface in response to various stimuli, e.g. during the Ca^{2+} spikes in the beating heart. It is important to emphasize that the Ca^{2+}-induced inhibition appears to be specific for the animal CcO. It is not observed with homologous bacterial CcO, even if the bacterial enzyme is mutated to a form that binds Ca^{2+} reversibly like the bovine oxidase. Such a specificity of the inhibitory action accords with the fact that Ca^{2+}-binding at the CBS involves two key residues in the exit part of the "proton channel H" revealed in the bovine heart CcO [2]. In the bacterial CcO the exit part of the H-proton pathway is not conserved. Thus, the selective inhibitory effect of Ca^{2+} on the bovine CcO supports a hypothesis on a special functional role of the H-channel in the animal CcO.

References

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S9.L4

The role of protein dynamics and thermal fluctuations in regulating cytochrome c/cytochrome c oxidase electron transfer

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Protein electron transfer (ET) reactions constitute the basis of energy transduction in living organisms and involve a variety of fine-tuning mechanisms that have just begun to be uncovered. In this respect, the notion of a "native" state associated with protein function is not sufficient for successfully describing intra- and inter-protein ET reactions. In this lecture I will present recent combined electrochemical, spectroelectrochemical, spectroscopic and computational studies on the ET reactions of cytochrome c and of the primary electron acceptor of cytochrome c oxidase, the CuA site, in biomimetic complexes. The results show that protein dynamics and thermal fluctuations allow these systems to explore different structural and electronic configurations that are crucial for their function, optimizing the kinetic parameters in different ways. Moreover, electrostatic interactions and local electric fields reshape the free energy surfaces that the proteins explore, in a manner that may be central for regulation of the respiratory electron transport chain. For example, the results reveal the existence of two native-like conformations of cytochrome c that present significantly different reorganization energy (λ). Conversion from the high to the low λ forms is triggered by electrostatic interactions, and involves the rupture of a weak H-bond between first- (M80) and second-sphere (Y67) ligands of the heme iron, as a distinctive feature of the conformational switch. The two flexible Ω loops operate as transducers of the electrostatic signal. On the other hand, the CuA site may populate two alternative ground states that differ in λ values and are optimized for electron entry and exit, respectively, through two different and nearly perpendicular pathways. The relative populations of these two redox active states can be modulated through first and second sphere mutations, through minor geometrical distortions and by application of biologically significant electric fields. The physiological relevance of these finding will be critically discussed in terms of a possible regulatory mechanism of the Cyt/CcO ET reaction in vivo.

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S9.O1

Proton gating in cytochrome c oxidase via D- and K-channels

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The central chemical reaction of aerobic metabolism is the conversion of molecular oxygen to water catalyzed by cytochrome c oxidase (CcO). In a stepwise redox-reaction, four electrons and four chemical protons enter the enzyme and are consumed in the reaction, while four physical protons are pumped across the membrane establishing an electro-chemical gradient. While all four electrons are transferred via the same cofactors to the binuclear reaction center (BNC), the protons take two different routes in the A-type CcO. Two of the four chemical protons arrive via the D-channel in the oxidative first half starting after oxygen binding. The other two chemical protons enter via the K-channel in the reductive second half of the reaction cycle. In contrast, all four pumped protons are transferred via the D-channel. This branched conduction of protons requires specific constraints onto the proton delivery channels. In this study, we use molecular dynamics simulation and electrostatic energy computations to analyze the proton channel properties and their gating elements that ensure unidirectional proton flux. The D- and the K-channels appear very different at first glance. The D-channel possesses a hydrogen bond network of water and several acidic groups connecting the aqueous phase with the BNC. In contrast, the hydrogen bond pattern of the K-channel is disconnected having only few titratable groups and little amount of water molecules in the crystal structure. Still, the channels share some similarities. Most impressively, both channels have in common a hydrophobic environment around their key residue — Glu286 (numbering from *Rhodobacter sphaeroides*) in the D-channel and Lys362 in the K-channel. As a consequence, these residues are likely in the charge-neutral protonation state, while glutamate and lysine are usually charged in aqueous solution. Therefore, change of protonation of Glu286 and Lys362 may be induced by changes in the hydration level or the electric field in a regulated manner. The unique feature of these key residues enables