Cytochrome P450 or high pH induce structural and functional changes of Coenzyme Q10

Rubin Gulaboski, Ivan Bogeski, Valentin Mirceski, Reinhard Kappl, Markus Hoth

Goce Delcev University, Stip, Macedonia, and Department of Biophysics, F,aculty of Medicine, University of Saarland, Homburg, Germany

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ABSTRACT

Coenzyme Q10 (CoQ10) is a lipid-soluble compound, indispensable for optimal functioning of all living organisms, and it is the only non-protein component of the mitochondrial electron-transport chain (ETC). Its primary function is to transfer electrons along the ETC and protons across the inner mitochondrial membrane (IMM). The concomitant proton gradient across the IMM is essential for ATP production. Cytochrome P450 (CYP450) monooxygenases are a large group of membrane bound enzymes. In humans, CYP450 are located mainly at the IMM or at the endoplasmic reticulum and are involved in conversion of a variety of substrates by catalysing diverse chemical reactions. Whether and how CoQ10 and CYP450 interact has been unknown.

Using voltammetry, UV-VIS spectrometry, electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) we analyzed the structural and functional changes of CoQ10 and its analog CoQ1 induced by CYP450 or by concentrated solution of sodium hydroxide (NaOH).

We show that both CYP450 and NaOH lead to cleavage of C-O bond of the methoxy (-O-CH_3) groups located at positions 2 and 3 on the quinone ring; a process known as O-demethylation. The newly created product has much stronger antioxidant properties than the native quinone and upon reduction is effectively chelating Ca^{2+} and other divalent cations. In addition, we found that the O-demethylated CoQ not only binds, but also transports Ca^{2+} across biomimetic artificial membranes. We currently investigate the physiological importance of our findings using yeast mitochondria lacking CoQ10 and mitochondria from humans with decreased levels of CoQ10.

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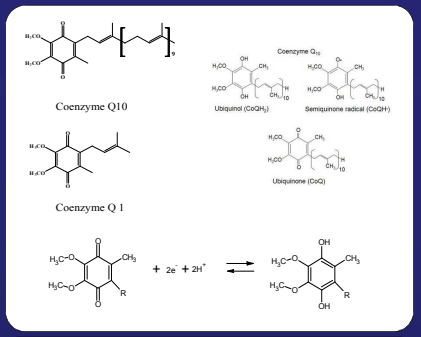

Upper panel: **Coenzyme Q structures considered**

Lower panel: **Scheme of the redox cycling between the "quinol" (oxidized form) and "quinol" (reduced form) that is typical for all Coenzyme Q family members in aqueous media.**

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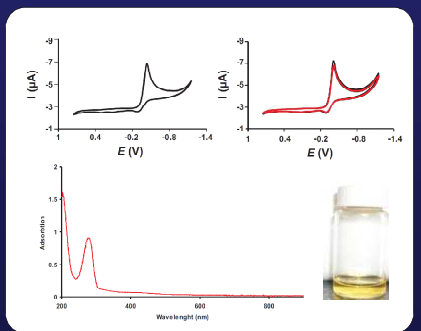

Upper panel: **Cyclic voltammogram showing the redox transformation of the native Coenzyme Q1 in pH of 7.00 (left), and the insensitivity of its redox transformation to the Ca^{2+} ions (red curves on the right voltammograms of upper panel are recorded in presence of 10 mM and higher concentration of Ca^{2+} ions).**

Lower panel: **UV-VIS spectrum of the native form of Coenzyme Q1 in pH of 7 (left), and the color of the solution of CoQ1 when dissolved in aquoeus neutral media (right)**

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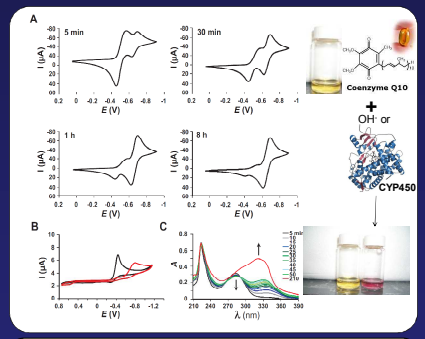

Panel A: **Cyclic voltammograms showing the chemical transformation of the native Coenzyme Q1 to another redox active form in strong alkaline solution;**

Panel B: **Comparison of cyclic voltamograms of the native form of Coenzyme Q1 (black curve), and the CoQ1 form that was obtained in alkaline media (red curve). Both voltammograms are recorded in pH of 7.00**

Panel C: **UV-VIS spectrum showing the chemical transformation of Coenzyme Q1 in alkaline media.**

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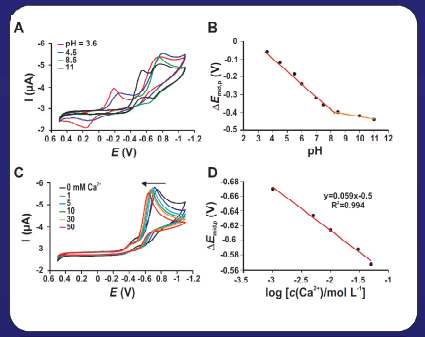

Panels A and B: **Cyclic voltammograms showing the pH dependence of the native Coenzyme Q1 form (peaks at more positive potentials) and the new form obtained in alkaline media (peak at more negative potentials). While the redox process of the native Coenzyme Q1 is sensitvite to pH (Panel B), the redox process of new form is insensitve to pH;**

Panels C&D: **Cyclic voltamograms showing the ability of the new form of Coenzyme Q1 to complexate Ca^{2+} ions. The stochiometry of that complex was 1:2 ($(L:M^{2+})$ (Panel D).**

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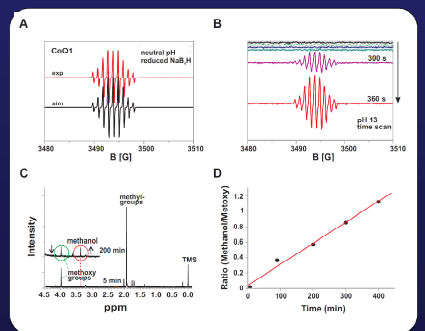

Panels A &B: **Electron Paramagnetic Spectra (EPR) of the radical of the native Coenzyme Q1, created upon its partial 1e- reduction with $NaBH_4$ in neutral media (A); EPR spectrum of Coenzyme Q1 obtained in 0.1 M NaOH, without adding a reductive mean (B). The radical on Panel B can be obtained only if a structure with CHARGE of „2-" is present in the system.**

Panels C&D: **NMR Spectrum of native Coenzyme Q1, and of the form of Coenzyme Q1 obtained after being in contact for 200 min with NaOH (C). The appearance of methanol and the concomitant decrease of the signal intensity of the both methoxy groups indicates scission of the two O-CH_3 groups under the influence of OH^- ions, pH of 7 in all cases.**

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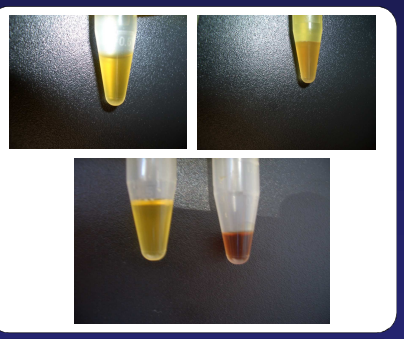

Upper Panel: **Coenzyme Q10 does not distribute at all between organic water immiscible solvent and water (left upper panel), but it does nicely between the same organic water immiscible solvent (1,2 dichlor ethan-DCE) and alkaline solution (right upper panel);**

Lower Panel: **Organic alkaline compounds (tetraoctylammonium hydroxide) and CYP450 enzyme induce dratsic change to the colour of the Coenzyme Q10 solution converting it from yellow to brownish (right snapshot of the lower panel)**

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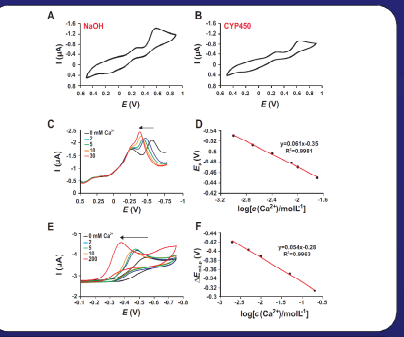

Panel A and B: **Cyclic voltammograms showing the chemical transformation of the native Coenzyme Q10 to another redox active form (the new peaks at more negative potentials) in strong alkaline solution (A) or in presence of P-450 Enzyme (B);**

Panel C and D: **Cyclic voltamograms showing that the form of Coenzyme Q10 formed in presence od CYP450 is able to bind Ca^{2+} ions (C) in 1:2 L to M stochiometry (D) upon its electrochemical reduction. These voltammograms are recorded in pH of 7.00**

Panel E and F: **The new form of CoQ10 can also transfer Ca^{2+} ions across biomimetic membranes upon its electrochemical reduction.**

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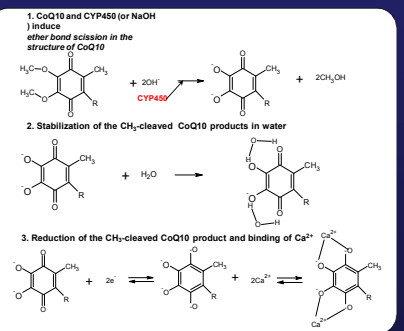

Simplified reaction scheme showing the scission of the etheric bonds of the two methoxy groups in the structure of Coenzyme Q10 in presence of NaOH or Cytochrome P-450 that leads to creation of the so-called double „O-demethylated" CoQ10 form. This form bears charge of „2-". (upper reaction scheme).

The lower reaction scheme shows the electrochemical reduction of the „O-demethylated" CoQ10 form and its consecutive complexation with the Ca^{2+} ions.

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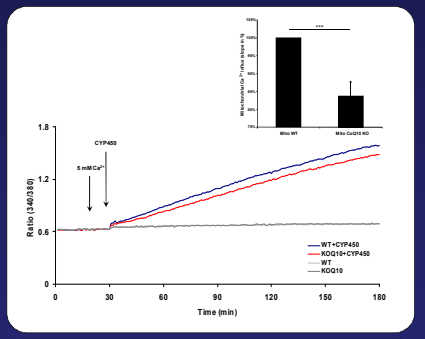

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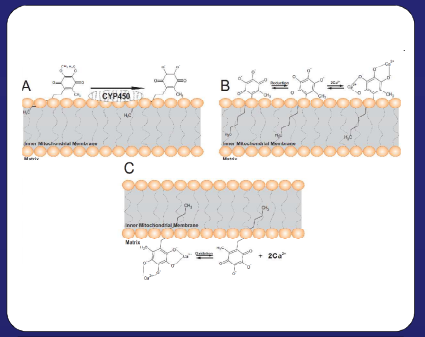

Panels A&B: **Sequence of the possible reactions that can lead to creation of O-demethylated CoQ10 form in the mitochondrial membranes, which is able to bind Ca^{2+} ions.**

Panel C: **The new negatively charged form of Coenzyme Q10 can facilitate the transfer of Ca^{2+} ions across the mitochondrial membrane.**

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SUMMARY

❑The chemistry and most of the functions of the native forms of Coenzyme Q family members are mainly portrayed in the features of the 2e-/2H⁺ redox reaction (electron and proton transfer) that leads to reversible transformation of the quinone to quinol forms.

❑If the Coezyme Q structures are in contact with high concentration of OH^- ions or CYP450 enzymes, quite different quinonic forms can be obtained.

❑CYP450 and NaOH can both induce scission of the both O-CH_3 (methoxy) groups in the structure of the Coenzyme Q family members, thus creating so called „O-demethylated" quinones that bear charge of „2-".

❑These new Coenzyme Q structures formed in alkaline media (or in presence of CYP450) are more polar than their parent compounds, while also having much stronger antioxidative features.

❑The inherent properties of the new Coenzyme Q structures to bind the earth-alkaline cations upon their reduction classify these compounds as potential facilitators for transferring of metal ions across biological membranes.