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REVIEW

The Multiple Functions of Coenzyme Q

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The coenzyme function of ubiquinone was subject of extensive studies in mitochondria since more than 40 years. The catalytic activity of ubiquinone (UQ) in electron transfer and proton translocation in cooperation with mitochondrial dehydrogenases and cytochromes contributes essentially to the bioenergetic activity of ATP synthesis. In the past two decades UQ was recognized to exert activities which differ from coenzyme functions in mitochondria. From extraction/reincorporation experiments B. Chance has drawn the conclusion that redox-cycling of mitochondrial ubiquinone supplies electrons for univalent reduction of dioxygen. The likelihood of $O_2^{\cdot -}$ release as normal byproduct of respiration was based on the existence of mitochondrial SOD and the fact that mitochondrial oxygen turnover accounts for more than 90% of total cellular oxygen consumption. Arguments disproving this concept are based on results obtained from a novel noninvasive, more sensitive detection method of activated oxygen species and novel experimental approaches, which threw light into the underlying mechanism of UQ-mediated oxygen activation. Single electrons for $O_2^{\cdot -}$ formation are exclusively provided by deprotonated ubisemiquinones. Impediment of redox-interaction with the bc_1 complex in mitochondria or the lack of stabilizing interactions with redox-partners are promotors of autoxidation. The latter accounts for autoxidation of antioxidant-derived ubisemiquinones in biomembranes, which do not recycle oxidized ubiquinols. Also $O_2^{\cdot -}$-derived H_2O_2 was found to interact with ubisemiquinones both in mitochondria and nonrecycling biomembranes when ubiquinol was active as antioxidant. The catalysis of reductive homolytic cleavage of H_2O_2, which contributes to HO· formation in biological systems was confirmed under defined chemical conditions in a homogenous reduction system. Apart from dioxygen and hydrogen peroxide we will provide evidence that also nitrite may chemically interact with the ubiquinol/bc_1 redox couple in mitochondria. The reaction product NO was reported elsewhere to be a significant bioregulator of the mitochondrial respiration and O_2 activation. Another novel finding documents the bioenergetic role of UQ in lysosomal proton intransport. A lysosomal chain of redox couples will be presented, which includes UQ and which requires oxygen as the terminal electron acceptor.

Key Words: ubiquinone, ubisemiquinone; mitochondria; lysosomes; liposomes; NO; ESR; oxygen radicals.

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INTRODUCTION

Coenzyme Q (ubiquinone, UQ)[1] is a unique biomolecule that is present in almost all biomembranes. Although its partition coefficient suggests a uniformly high concentration in the lipid phase of cell- and organelle membranes it is not distributed according to physical solvatation properties (*1*,*2*). For instance ubiquinone is a major constituent of the inner mitochondrial membrane, the Golgi vesicles, and lysosomal membranes, while only few molecules of UQ are found in the membrane of LDL particles (*1*). This unexpected distribution suggests UQ has quite different functions in the various biological membrane systems.

From the chemical properties of UQ a function in electron transfer and proton translocation can be expected. The stepwise addition of electrons and protons gives rise to various UQ metabolites all differing in their polarities. As a consequence UQ undergoing redox-cycling are expected to spread across all sections of a bilayer supporting anisotropic distribution of protons. These redox properties open a broad spectrum of biochemical activities. It is well established that the major activity of UQ in the mitochondrial inner membrane is its participation in energy-linked respiration (*3*–*5*). Redox-cycling of UQ between dehydrogenases and cytochromes not only drives protons from the matrix to the cytosolic membrane side it was also suggested to provide single electrons for electron acceptors which do not form part of the respiratory chain.

The direct transfer of single electrons to dioxygen from redox-cycling UQ was long suggested to be responsible for the existence of radicals in the cell. In contrast to this prooxidant function that was assumed to result from autoxidation of the univalently reduced UQ species the divalently reduced UQ species is considered to control lipid peroxidation by recycling α-tocopherol radicals to the antioxidant active form and proposed to have an SOD-like function (*6*).

The present study critically analyses the pro- and antioxidant role of coenzyme Q (*7*,*8*) and provides experimental evidence on novel functions such as the mitochondrial nitroxide metabolism as well as the contribution to lysosomal proton translocation.

MATERIAL AND METHODS

Ubiquinone-10 was obtained from Kanegafuchi Chemical Industry Co. LTD. DMPO was purchased from Sigma. Other chemicals were obtained from Merck. All reagents were of the highest grade of purity.

Mitochondria were prepared from hearts of male Sprague–Dawley rats (250–300 g) by differential centrifugation of a cell homogenate according to Szarkowska and Klingenberg (*9*). Following preparation the quality of mitochondria was monitored by measurement of respiratory control values and ATP/oxygen ratios using a Clark-type electrode. For the measurement of ubisemiquinone populations during respiration

[1] Abbreviations used: AA, antimycin A; CumOOH, cumol hydroperoxide; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetate; ESR, electron spin resonance; LDL, low density lipoprotein; RHM, rat heart mitochondria; RLM, rat liver mitochondria; SOD, superoxide dismutase; Tris, tris(hydroxymethyl)-aminomethane; UQ, ubiquinone; $UQ\cdot^{-}$, ubisemiquinone; UQH_2, ubiquinol.

under anaerobic conditions oxygen was replaced by ferricyanide, which accepts electrons from reduced cytochrome c in mitochondria. Enrichment of toluene in mitochondrial membranes was achieved by incubation with toluene-saturated buffers for 10 min at 4°C giving final concentrations of about 200 nmol toluene/mg of mitochondrial protein. For the incorporation of cholesterol into mitochondrial membranes 300 μl of submitochondrial particles (SMP, 20 mg of protein/ml) were lyophilized yielding about 40 mg of dried lyophilisate. Each 20 mg of lyophilisate were mixed with 500 μl of pentane, 1.5 μl of ethanol, and 2.44 μmol of cholesterol. After thorough mixing, organic solvents were removed in a stream of argon and SMP were dried in vacuum. Afterwards samples were resuspended in 200 μl of water. Lysosomes were obtained from a light mitochondrial fraction of a rat liver homogenate of male Sprague–Dawley rats. Separation of lysosomes from other cellular organelles was achieved by centrifugation in a self-generating gradient of iodixanol according to Graham *et al.* (*10*,*11*). The purity of lysosomal fractions was assessed from the activity of organelle-specific marker enzymes; acid phosphatase (lysosomes), succinate dehydrogenase (mitochondria), and cyanide-insensitive NADH:cytochrome c reductase (microsomes) (*12*).

ESR measurements were performed on a Bruker ESP300 spectrometer equipped with an X-band microwave source. Photometric measurements of organelle suspensions were carried out on a dual-wavelength spectrometer SLM Aminco DW2000.

RESULTS AND DISCUSSION

Identity of O_2-Reducing UQ Species

Reduction of UQ yields one protonated and one deprotonated ubisemiquinone species, a fully, a semi- and a nonprotonated divalently reduced ubiquinone. Divalently reduced ubiquinones can be obtained chemically using borohydride as the reductant and semiquinones are formed when oxidized UQ is mixed with UQH_2 according to reaction.

$$UQH_2 + UQ \rightleftharpoons UQ\cdot^- + UQ\cdot^- + 2H^+ \qquad [1]$$

Protonation or deprotonation of semi- or divalently reduced UQ is a function of the pH value established (*13*). The widely differing pK values of semi- and divalently reduced UQ in combination with comproportionation allow to concentrate each of the five UQ species separately and study their stability in the absence and presence of oxygen (*14*). The results exclude all three divalently reduced ubiquinones as reductants of oxygen; only deprotonated ubisemiquinones destabilize when oxygen was added (Fig. 1).

Autoxidation of Mitochondrial Ubisemiquinones

In mitochondria ubisemiquinones are mainly in the deprotonated form (*15*). This is to be expected since the pK ranges far below the physiological pH (*16*). However, ESR signals obtained from redox-cycling ubisemiquinones remain unchanged in the presence of oxygen (Fig. 2) (*17*). Different from ubisemiquinones in homogenous reaction systems ubisemiquinones in mitochondrial membranes are stabilized upon

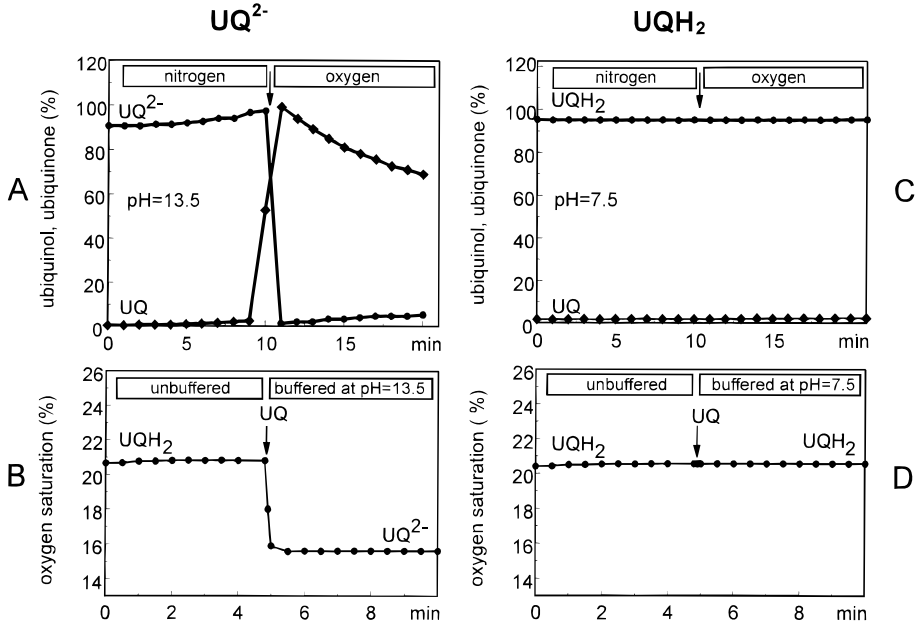

FIG. 1. Effect of pH on the oxidation of ubiquinol in the presence of molecular oxygen. Oxidation of the ubiquinol dianion and oxygen consumption following the addition of oxygen **(A)** and the addition of oxidized ubiquinone to drive semiquinone formation **(B)** at pH 13.5. **(C)** and **(D)** are analogous experiments performed at pH 7.5. Experimental conditions **(A)** and **(C):** UQH_2 (final concentration) was mixed with ethanol, containing 1% H_2O and 2.5 mM Tris (pH 7.5 or 13.5) in the absence of oxygen. To start ubiquinol oxidation the solution was equilibrated with air oxygen (indicated by an arrow). **(B** and **D):** Unbuffered ethanol solution of UQH_2 (final conc. 300 μM) was mixed (indicated by an arrow) with UQ (final conc. 300 μM) buffered at pH 7.5 or 13.5 in the presence of air oxygen (for further details see Kozlov *et al.* (*14*)).

physical interactions with the respective redox partners. Destabilization of mitochondrial ubisemiquinones occurs when the basic molecular structure of the phospholipid bilayer was deranged.

Irrespectively whether order parameters defined from spin labeling experiments were decreased (incorporation of toluene) or increased (incorporation of cholesterol) ubisemiquinone pools detectable at room temperature were sensitive to oxygen and O_2 radicals were released from these mitochondria (Fig. 3) (*17*). Thus, it appears that changes of the order parameters of the lipid bilayer affect the regular electron transfer from ubisemiquinone to its oxidant, the bc_1 complex.

The complexity of electron bifurcation from redox-cycling UQ to the bc_1 complex renders the proper control of electron transfer particularly susceptible (Fig. 4). Since the conformational change of the bc_1 complex is a prerequisite to overcome thermodynamic restraints a hindrance of adequate protein arrangement will render regular electron transfer more difficult. Such a situation is expected to facilitate branching off electrons to nonphysiological acceptors like dioxygen. Autoxidation of ubisemiquinones indicates an impediment of regular ubisemiquinone oxidation by the bc_1 complex

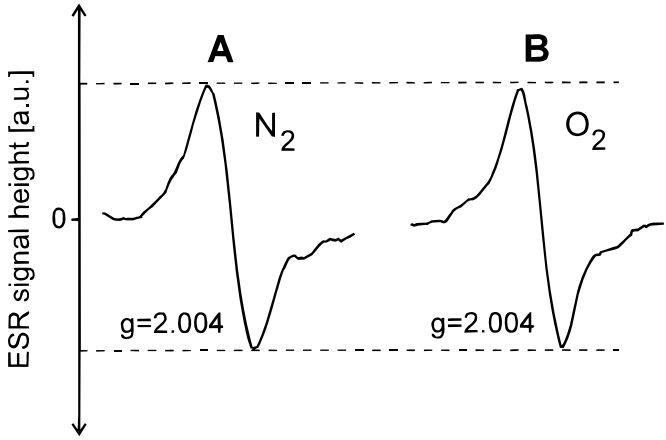

FIG. 2. Insensitivity of mitochondrial ubisemiquinones to dioxygen. ESR signal of $UQ\cdot^{-}$ in succinate-respiring mitochondria **(A)** under anaerobic conditions, **(B)** in the presence of oxygen. Oxygen was replaced in **(A)** by ferricyanide as mitochondrial electron acceptor and mitochondria were kept under nitrogen. The incubation mixture contained 3.2 mg/ml RLM, 24 mM fumarate/succinate 1:5, and 7.3 mM phosphate, and 12 mM $Fe(CN)_6^{3-}$ if required. Immediately after the addition of ferricyanide and succinate/fumarate (20/4 mM) the reaction mixture, which was placed in a quartz tube, was frozen by liquid nitrogen and subjected to ESR measurements. ESR settings: microwave power 9.46 GHz; frequency 1 mW; modulation frequency 100 KHz; modulation amplitude 5G; receiver gain $2\cdot10^4$.

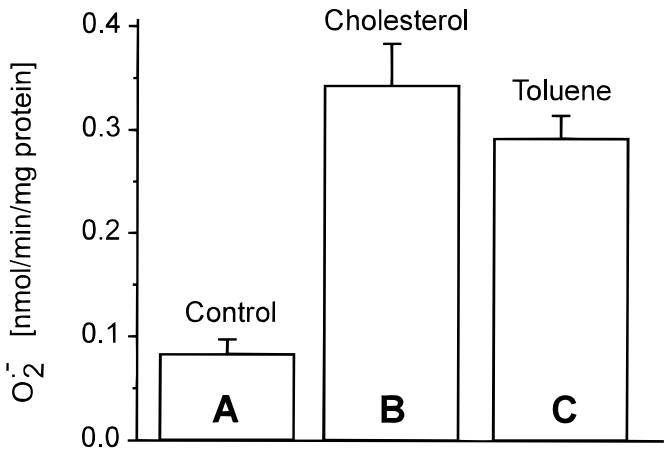

FIG. 3. Conditions resulting in $O_2\cdot^{-}$ release during respiration. Mitochondrial respiration was initiated upon the addition of succinate (final concentration 20 mM). Control experiments **(A)** with nontreated mitochondria stored under the same conditions as (**B** and **C**). Mitochondria preloaden with cholesterol **(B)** or mitochondria preloaden with toluene (30 min, r.t.) **(C)** (*20*). Superoxide formation was assayed by SOD-sensitive reduction of acetylated cytochrome c (*30*).

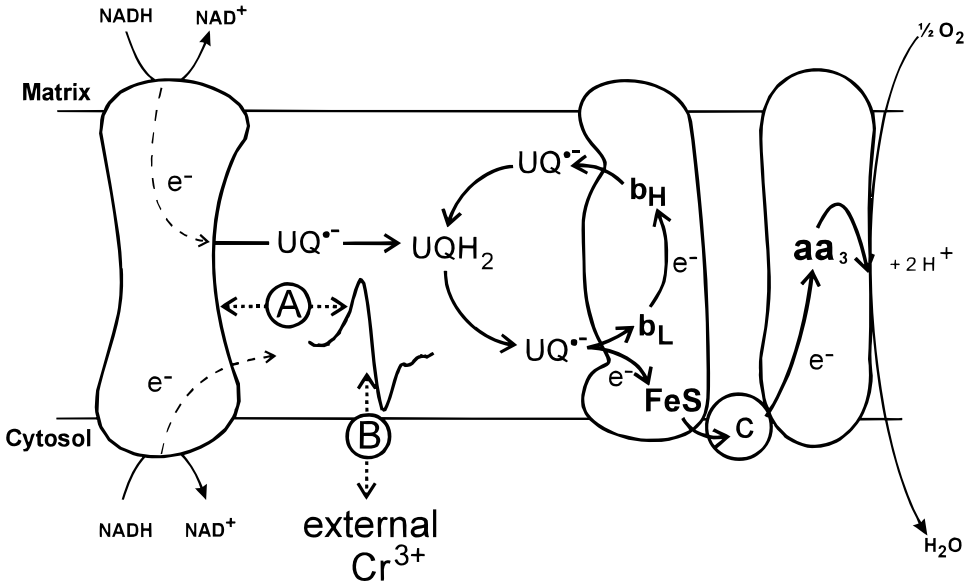

FIG. 4. Schematic presentation of the mitochondrial respiratory chain (heart mitochondria). NADH from citric acid cycle or from the cytosol supplies reducing equivalents for respiration via the endogenous or exogenous NADH dehydrogenase of complex I. Ubisemiquinones ($UQ\cdot^{-}$) enter the UQH_2 pool which drives the $UQ\cdot^{-}$–cytochrome b_L–cytochrome b_H cycle and diverges single electrons to cytochrome aa_3 via the Rieske iron sulfur protein (FeS) and cytochrome c (c). Cytosolic NADH activates a ubisemiquinone species (ESR spectrum inserted), which interacts with complex I **(A)** and operates dense to the bordering water phase **(B)** as detected by the interaction with an hydrophilic paramagnetic Cr^{3+} salt. For details see (*20*).

in membranes in which hydrophobic/hydrophilic interactions were affected through changes of the phospholipid order.

Heart mitochondria were earlier shown to directly oxidize external NADH when physiological levels are exceeded (*18*). For instance cytosolic NADH increases five to seven fold in ischemic organs due to anaerobic glycolysis.

NADH has access to the exogenous NADH dehydrogenase, an enzyme shuttling reducing equivalents into the respiratory chain via complex I (see Fig. 4). Electron transfer occurs along all components of the respiratory chain ultimately reducing oxygen via cytochrome oxidase to water. In contrast to respiration of endogenous substrates protons required for water formation are taken from the outside thereby decreasing the transmembraneous proton gradient otherwise used for ATP synthesis (*19*). This particular non-energy-linked electron transfer pathway includes a ubisemiquinone species functionally in contact with complex I (*20*). ESR characteristics reveal a relatively low spin-lattice interaction with its redox partners, which became evident from low microwave saturation conditions and in addition localization close to the polar head group section of the membrane was observed. The latter was inferred from spin coupling of an external spin probe (Cr^{3+}) with the paramagnetic center of the