

Evaluation of the Antioxidant Capacity of Ubiquinol and Dihydrolipoic Acid*

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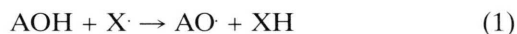
Ubiquinol, Coenzyme Q, Dihydrolipoic Acid, Lipid Peroxidation, Oxygen Radicals

Ubiquinone and α -lipoic acid are natural constituents which are involved in mitochondrial energy metabolism. Their bioenergetic activities require redox-cycling. In the case of α -lipoic acid redox-cycling leads to dihydrolipoic acid which occurs in multienzyme complexes involved in the citric acid cycle while UQ recycles through semi- and divalently reduced ubiquinones in the respiratory chain. We have proved the validity of the concept about the antioxidant function of these natural compounds in their reduced form. Ubiquinol was found to interfere with lipid peroxidation of liposomal membranes being itself degraded by two consecutive oxidation steps. Dihydrolipoic acid was found to totally recycle ubiquinone to the antioxidant active divalently reduced form. In contrast to the antioxidative derived reaction products of ubiquinol which in turn promoted lipid peroxidation, the antioxidant derived reaction product of dihydrolipoic acid was the unreactive two electron oxidation product α -lipoic acid. Our experiments demonstrate the existence of an dihydrolipoic acid driven recycling of UQ to the antioxidative-active UQH₂. The efficiency of the antioxidative capacity of the latter was found to be diminished through prooxidant activities of the antioxidant-derived metabolites.

Introduction

In recent literature ubiquinol (UQH₂, reduced Coenzyme Q) and dihydrolipoic acid (DHLA) are reported to exert antioxidant functions besides their role in mitochondrial energy metabolism. It is believed that these compounds are of major significance as natural antioxidants since in contrast to other biological antioxidants, such as α -tocopherol (vitamin E), or β -carotene their capacity cannot become exhausted as a result of oxidative stress (Kagan *et al.*, 1990; Thomas *et al.*, 1996). However, the reactivity of the antioxidant-derived reaction product may counteract these positive properties of DHLA and UQH₂, a fact which has not yet been sufficiently considered. According to

the general equation describing the interaction of an antioxidant (AOH) with a radical (X $\cdot$)



it should be expected that UQH₂ refills ubisemiquinone (SQ $\cdot^-$) pools when acting as an antioxidant while DHLA yields thiyl radicals. Both radical species have been reported to act as prooxidants in biological systems (Nohl *et al.*, 1996; DeGray and Mason, 1995). Furthermore, it is far from being clear whether UQH₂ can exert important antioxidant functions in biomembranes which have no recycling systems for ubiquinone (UQ), such as LDL (low density lipoprotein) particles. We believe that the antioxidant efficiency of UQH₂ and DHLA is a variable value being dependent on: 1. The chemical potency of the antioxidant-derived reaction product. 2. The existence and efficiency of a recycling system which transforms the antioxidant-derived reaction product back to its antioxidant form. In the present study we, therefore, analyzed reaction products evolving from the antioxidant activity of UQH₂ and DHLA and the fate of these products in their natural environment.

Abbreviations: UQ, ubiquinone; UQH₂, ubiquinol; SQ $\cdot^-$, ubisemiquinone. No side chain is indicated by the index "0"; natural UQ containing the isoprenic side chain has the index "10". DHLA, dihydrolipoic acid.

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Results and Discussion

DHLA and UQH₂ were shown to reestablish vitamin E following one-electron oxidation to the chromanoxyl radical. Since in biological membranes the probability of a collision between lipophilic antioxidants and lipid radicals is much higher as compared to an interaction with chromanoxyl radicals the antioxidant activity of DHLA and UQH₂ is unlikely to be restricted to scavenging reactions with vitamin E radicals only. We, therefore, studied the efficiency of UQ₁₀H₂ and DHLA in scavenging peroxy radicals in a homogenous system (acetonitrile) using photolytic cleavage of AIBN (azobis-isobutyronitrile) as the peroxy radical source. The results elucidate that UQ₁₀H₂ reacts as effective with these organic radicals as α -tocopherol while DHLA is about one magnitude less active. Starting from the idea that the net antioxidant activity is also dependent on the nature of the antioxidant derived reaction product the experiment with UQ₁₀H₂ was repeated in the more natural environment of a phospholipid bilayer where lipid peroxy radicals were formed as a result of lipid peroxidation (LPO) (Fig. 1).

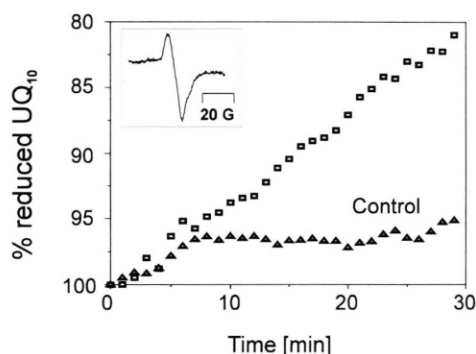


Fig. 1. Reoxidation of UQ₁₀H₂ in DOPC liposomes during LPO initiated by UV irradiation of AIBN in presence of air. Control samples were irradiated in absence of AIBN. (DOPC, dioleoyl phosphatidylcholine; AIBN, azobis-isobutyronitrile).

UQ₁₀H₂ was totally oxidized to UQ₁₀ shortly after LPO was initiated (Fig. 1). Under these conditions a transient ESR single line spectrum was observed which could be assigned on the basis of ESR characteristics to the existence of a SQ₁₀^{•−} species ($g = 2.005$, $\Delta H_{pp} = 8.9$ G at 200 K). Since we have earlier shown that the stability of SQ₁₀^{•−} decreases when approaching the polar-head group

section of the bilayer (Nohl *et al.*, 1996) the localization of this radical intermediate within the lipid membrane was studied by spin-exchange experiments with a water-soluble gadolinium salt. Spin-exchange is not expected with SQ₁₀^{•−} in the lipophilic inner section of the bilayer, while SQ₁₀^{•−} existing in the polar-head group phase should interact. The ESR signal amplitude was twice as high in the presence of gadolinium under saturation conditions indicating a portion of SQ₁₀^{•−} was accessible from the aqueous phase. Under these conditions autooxidation of SQ₁₀^{•−} is thermodynamically favored giving rise to the release of superoxide radicals. Therefore, the antioxidant activity of UQ₁₀H₂ can be directly linked to subsequent O₂^{•−} formation. The molar ratio between removal of organic radicals and the subsequent generation of O₂^{•−} is governed by the polarity of the surrounding of SQ₁₀^{•−} evolving from the antioxidant function of UQ₁₀H₂. Since the polarity of a membrane increases with the progression of LPO the antioxidant capacity of UQ₁₀H₂ can be expected to change from protection of LPO to stimulation of oxidative stress. The antioxidant activities of DHLA were investigated with a great variety of radical species. Until now no attention was devoted however, on the interaction between DHLA and UQ. Such an interaction is of particular interest in biomembranes which do not have recycling systems to maintain UQ in the antioxidant form (p.e. LDL). In a homogenous aqueous reaction system DHLA was found to totally reduce UQ₀ in an equimolar stoichiometry. Although this stoichiometry suggested a two electron reduction step SQ₀^{•−}-related ESR signals were observed (Schönheit *et al.*, 1995). Two possibilities were considered to explain SQ₀^{•−} formation:

1. UQ₀ + DHLA (HS-SH) →
SQ₀^{•−} + H⁺ + ·S-SH (thiyl radical) (2)
2. UQ₀H₂ + UQ₀ ↔ 2 SQ₀^{•−} + 2 H⁺
(comproportionation) (3)

SQ₀^{•−} formation following reaction (2) should be linked to the existence of thiyl radicals. However, thiyl radicals were not detectable. Reaction (3) was studied by the kinetic analysis of UQ₀H₂ and SQ₀^{•−} generation (Fig. 2). UQ₀H₂ formation occurred rapidly after the addition of DHLA. SQ₀^{•−}-related ESR signals ($g = 2.005$, $a_{3H(5CH_3)} = 2.4$ G, $a_{1H(6H)} = 2.1$ G at 293 K) became only detectable

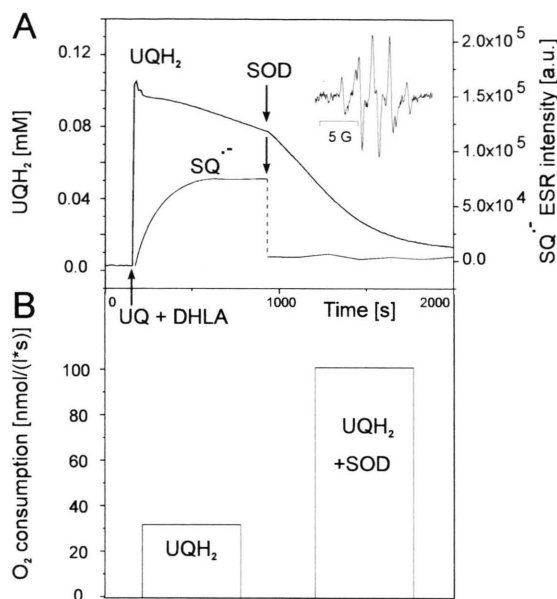
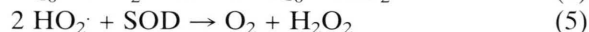
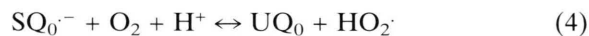


Fig. 2. Kinetics of UQ₀ reduction by DHLA followed by spontaneous and SOD-enhanced reoxidation of UQ₀H₂ in an aqueous environment. (A) Upper trace: time course of UQ₀H₂ concentration. Lower trace: ESR intensity of SQ^{•-} radicals. (B) Oxygen consumption during spontaneous and SOD-enhanced autoxidation of UQ₀H₂. (DHLA, dihydrolipoic acid; SOD, superoxide dismutase; UQ₀, ubiquinone 0).

when UQ₀H₂ formation was finished during the spontaneous reoxidation of UQ₀H₂. In contrast to the reduction process of UQ₀ SQ^{•-} formation was linked to oxygen consumption. SOD was found to stimulate both oxygen consumption and reoxidation of UQ₀H₂ while SQ^{•-}-related ESR-signals disappeared. We believe that SQ^{•-} are derived from comproportionation of UQ₀H₂ (according to reaction (3)). Due to the presence of protons SQ^{•-} can undergo autoxidation (reaction (4)) elucidating the consumption of oxygen. The equilibrium shift induced in the presence of SOD reveals the involvement of O₂^{•-} radicals (reaction (5)).



Since SOD deranges the stationary system by removing O₂^{•-} radicals from the equilibrium the steady state concentration of the intermediate SQ^{•-} decreases under the detection level (reaction (4)). We conclude from these findings that DHLA is able to reduce UQ₀ by a two-electron transfer

step to UQ₀H₂ which subsequently equilibrates via comproportionation with UQ₀ and SQ^{•-}. In Fig. 3 we studied whether these interactions between DHLA and UQ observed will also proceed in liposomes preloaded with UQ₁₀. After half an hour DHLA was fully oxidized while UQ₁₀ was reduced to UQ₁₀H₂. Since the reduction rate of UQ₁₀ in liposomes by DHLA from the aqueous phase is rather small we believe that shuttling of reducing equivalents from DHLA to UQ₁₀ in LDL particles is not likely to play a major role in maintaining the antioxidant capacity of UQ₁₀H₂.

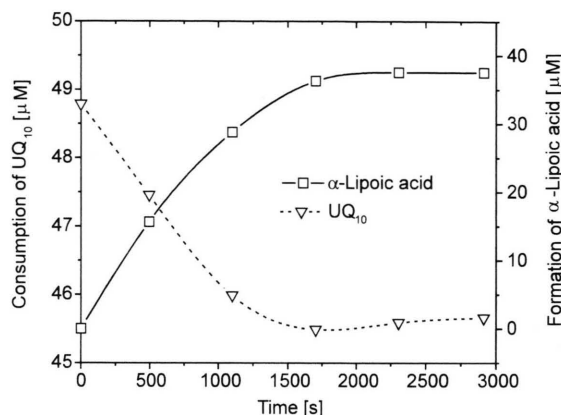


Fig. 3. Reduction of UQ₁₀ within the liposomal bilayer (DMPC, dimyristoyl phosphatidylcholine) by DHLA added. (DHLA, dihydrolipoic acid; UQ₁₀, ubiquinone 10).

These experimental observations suggest that the antioxidant efficiency of UQH₂ in biological membranes is dependent on: (i) The fate of the first antioxidant-derived product (SQ^{•-}) which may counteract the antioxidant activity by O₂ radical formation. (ii) Recycling of the second oxidant-derived product (UQ) to the antioxidant form (UQH₂) which increases the antioxidant capacity.

The antioxidant capacity may be impaired or totally suspended when SQ^{•-} formed undergo autoxidation. Since autoxidation of SQ^{•-} requires protons the balance is positive if SQ^{•-} evolving from antioxidant activities of UQH₂ exist in the apolar membrane phase and negative if SQ^{•-} exist in the polar-head group section. Physical membrane alteration resulting from LPO are expected to stimulate the prooxidant activity of SQ^{•-}. Thus, the antioxidant activity may change during oxidative stress. In biological membranes such as LDL particles which have no proper recycling systems the

antioxidant capacity is related to UQH₂ concentration and the balance between the latter and the fate of the antioxidant-derived SQ⁻ radicals. DHLA was found to be capable of recycling UQ in non-recycling biological membranes.

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