Evaluation of the Antioxidant Capacity of Ubiquinol and Dihydrolipoic Acid*

H. Nohl* and L. Gille

Institute of Pharmacology and Toxicology, Veterinary University Vienna, Veterinärpl. 1, A-1210 Vienna, Austria

Z. Naturforsch. **53c,** 250–253 (1998); received December 19, 1997/February 3, 1998

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

Ubiquinone and α-lipoic acid are natural constitutents 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 degradated 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 ubiquinols 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 UQH_2. 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 (UQH_2, 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 UQH_2, a fact which has not yet been sufficiently considered. According to the general equation describing the interaction of an antioxidant (AOH) with a radical ($X^{\cdot}$)

$$AOH + X^{\cdot} \rightarrow AO^{\cdot} + XH \qquad (1)$$

it should be expected that UQH_2 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 UQH_2 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 UQH_2 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 UQH_2 and DHLA and the fate of these products in their natural environment.

Abbreviations: UQ, ubiquinone; UQH_2, 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.

* This contribution was presented at a Workshop on the occasion of the 20th anniversary of the “Oxygen Club Munich”, organized by M. Saran and E. F. Elstner.

Reprint requests to Prof. Hans Nohl.
Fax: +43 1 25077 4490.
E-mail: hans.nohl@vu-wien-ac.at.

Results and Discussion

DHLA and UQH_2 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 UQH_2 is unlikely to be restricted to scavenging reactions with vitamin E radicals only. We, therefore, studied the efficiency of $UQ_{10}H_2$ and DHLA in scavenging peroxyl radicals in a homogenous system (acetonitrile) using photolytic cleavage of AIBN (azobis-isobutyronitrile) as the peroxyl radical source. The results elucidate that $UQ_{10}H_2$ 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 $UQ_{10}H_2$ was repeated in the more natural environment of a phospholipid bilayer where lipid peroxyl radicals were formed as a result of lipid peroxidation (LPO) (Fig. 1).

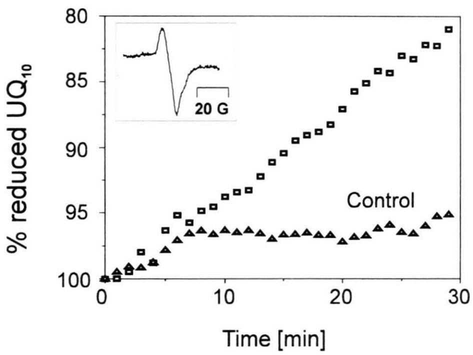

Fig. 1. Reoxidation of $UQ_{10}H_2$ in DOPC liposomes during LPO initiated by UV irradiation of AIBN in presence of air. Control samples were irradiated in absence of AIBN. (DOPC, dioleyl phosphatidylcholine; AIBN, azobis-isobutyronitrile).

$UQ_{10}H_2$ was totally oxidized to UQ_{10} 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 $SQ_{10}^{\cdot -}$ species (g = 2.005, ΔH_{pp} = 8.9 G at 200 K). Since we have earlier shown that the stability of $SQ_{10}^{\cdot -}$ 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 $SQ_{10}^{\cdot -}$ in the lipophilic inner section of the bilayer, while $SQ_{10}^{\cdot -}$ 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 $SQ_{10}^{\cdot -}$ was accessible from the aqueous phase. Under these conditions autoxidation of $SQ_{10}^{\cdot -}$ is thermodynamically favored giving rise to the release of superoxide radicals. Therefore, the antioxidant activity of $UQ_{10}H_2$ can be directly linked to subsequent $O_2^{\cdot -}$ formation. The molar ratio between removal of organic radicals and the subsequent generation of $O_2^{\cdot -}$ is governed by the polarity of the surrounding of $SQ_{10}^{\cdot -}$ evolving from the antioxidant function of $UQ_{10}H_2$. Since the polarity of a membrane increases with the progression of LPO the antioxidant capacity of $UQ_{10}H_2$ 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 UQ_0 in an equimolar stoichiometry. Although this stoichiometry suggested a two electron reduction step $SQ_0^{\cdot -}$-related ESR signals were observed (Schönheit *et al.,* 1995). Two possibilities were considered to explain $SQ_0^{\cdot -}$ formation:

1. UQ_0 + DHLA (HS-SH) → $SQ_0^{\cdot -}$ + H^+ + ·S-SH (thiyl radical) (2)
2. $UQ_0H_2 + UQ_0 \leftrightarrow 2\ SQ_0^{\cdot -} + 2\ H^+$ (comproportionation) (3)

$SQ_0^{\cdot -}$ 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 UQ_0H_2 and $SQ_0^{\cdot -}$ generation (Fig. 2). UQ_0H_2 formation occurred rapidly after the addition of DHLA. $SQ_0^{\cdot -}$-related ESR signals (g = 2.005, $a_{3H(5CH3)}$ = 2.4 G, $a_{1H(6H)}$ = 2.1 G at 293 K) became only detectable

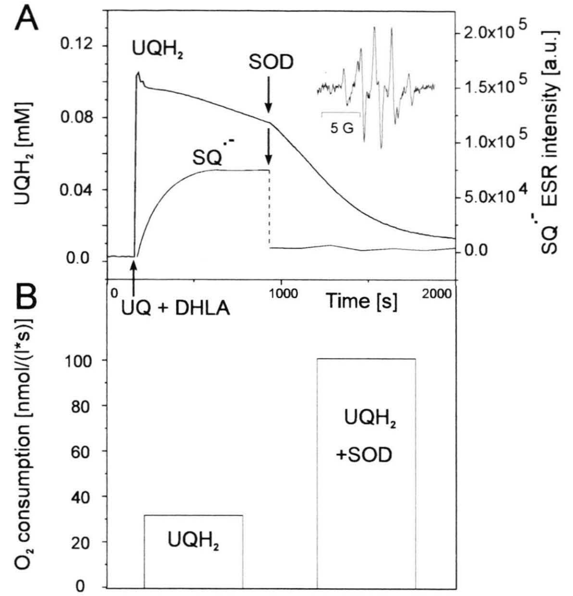

Fig. 2. Kinetics of UQ_0 reduction by DHLA followed by spontaneous and SOD-enhanced reoxidation of UQ_0H_2 in an aqueous environment. (A) Upper trace: time course of UQ_0H_2 concentration. Lower trace: ESR intensity of $SQ^{\cdot-}$ radicals. (B) Oxygen consumption during spontaneous and SOD-enhanced autoxidation of UQ_0H_2. (DHLA, dihydrolipoic acid; SOD, superoxide dismutase; UQ_0, ubiquinone 0).

when UQ_0H_2 formation was finished during the spontaneous reoxidation of UQ_0H_2. In contrast to the reduction process of UQ_0 $SQ_0^{\cdot-}$ formation was linked to oxygen consumption. SOD was found to stimulate both oxygen consumption and reoxidation of UQ_0H_2 while $SQ_0^{\cdot-}$-related ESR-signals disappeared. We believe that $SQ_0^{\cdot-}$ are derived from comproportionation of UQ_0H_2 (according to reaction (3)). Due to the presence of protons $SQ_0^{\cdot-}$ can undergo autoxidation (reaction (4)) elucidating the consumption of oxygen. The equilibrium shift induced in the presence of SOD reveals the involvement of $O_2^{\cdot-}$ radicals (reaction (5)).

$$SQ_0^{\cdot-} + O_2 + H^+ \leftrightarrow UQ_0 + HO_2^{\cdot} \quad (4)$$
$$2\ HO_2^{\cdot} + SOD \rightarrow O_2 + H_2O_2 \quad (5)$$

Since SOD deranges the stationary system by removing $O_2^{\cdot-}$ radicals from the equilibrium the steady state concentration of the intermediate $SQ^{\cdot-}$ decreases under the detection level (reaction (4)). We conclude from these findings that DHLA is able to reduce UQ_0 by a two-electron transfer step to UQ_0H_2 which subsequently equilibrates via comproportionation with UQ_0 and $SQ_0^{\cdot-}$. In Fig. 3 we studied whether these interactions between DHLA and UQ observed will also proceed in liposomes preloaded with UQ_{10}. After half an hour DHLA was fully oxidized while UQ_{10} was reduced to $UQ_{10}H_2$. Since the reduction rate of UQ_{10} in liposomes by DHLA from the aqueous phase is rather small we believe that shuttling of reducing equivalents from DHLA to UQ_{10} in LDL particles is not likely to play a major role in maintaining the antioxidant capacity of $UQ_{10}H_2$.

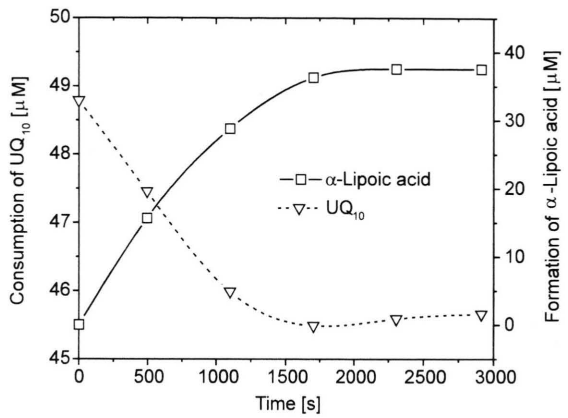

Fig. 3. Reduction of UQ_{10} within the liposomal bilayer (DMPC, dimyristoyl phosphatidylcholine) by DHLA added. (DHLA, dihydrolipoic acid; UQ_{10}, ubiquinone 10).

These experimental observations suggest that the antioxidant efficiency of UQH_2 in biological membranes is dependent on: (i) The fate of the first antioxidant-derived product ($SQ^{\cdot-}$) which may counteract the antioxidant activity by O_2 radical formation. (ii) Recycling of the second oxidant-derived product (UQ) to the antioxidant form (UQH_2) which increases the antioxidant capacity.

The antioxidant capacity may be impaired or totally suspended when $SQ^{\cdot-}$ formed undergo autoxidation. Since autoxidation of $SQ^{\cdot-}$ requires protons the balance is positive if $SQ^{\cdot-}$ evolving from antioxidant activities of UQH_2 exist in the apolar membrane phase and negative if $SQ^{\cdot-}$ exist in the polar-head group section. Physical membrane alteration resulting from LPO are expected to stimulate the prooxidant activity of $SQ^{\cdot-}$. 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 UQH_2 concentration and the balance between the latter and the fate of the antioxidant-derived $SQ^{\cdot -}$ radicals. DHLA was found to be capable of recycling UQ in non-recycling biological membranes.

Acknowledgement

The present study was supported by the Austrian Fonds zur Förderung der wissenschaftlichen Forschung (P11447-MED).

DeGray J. A. and Mason R. P. (1995), Biothiyls: Free Radical Chemistry and Biological Significance., Biothiols in Health and Disease (eds. L. Packer, E. Cadenas), Marcel Dekker, New York, 65–81.

Kagan V., Serbinova E. and Packer L. (1990), Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. Biochem. Biophys. Res. Comms. **169,** 851–857.

Thomas S. R., Neuzil J. and Stocker R. (1996), Cosupplementation with coenzyme Q prevents the prooxidative effect of alpha-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. Arterioscl. Throm. Vasc. Biol. **16,** 687–696.

Nohl H., Gille L., Schönheit K. and Liu Y. (1996), Conditions allowing redox-cycling ubisemiquinone in mitochondria to establish a direct redox couple with molecular oxygen. Free Radic. Biol. Med. **20,** 207–213.

Schönheit K., Gille L. and Nohl H. (1995), Effect of alpha-lipoic acid and dihydrolipoic acid on ischemia/reperfusion injury of the heart and heart mitochondria. Biochim. Biophys. Acta **1271,** 335–342.