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Inhibition of lipid peroxidation by ubiquinol in submitochondrial particles in the absence of vitamin E

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The relationship between the antioxidant effects of reduced coenzyme Q_{10} (ubiquinol, UQH_2) and vitamin E (α-tocopherol) was investigated in beef heart submitochondrial particles in which lipid peroxidation was initiated by incubation with ascorbate+ADP-Fe^{3+}. These effects were examined after extraction of coenzyme Q_{10} (UQ-10) and vitamin E from the particles and reincorporation of the same components alone or in combination. The results show that UQH_2 efficiently inhibits lipid peroxidation even when vitamin E is absent. It is concluded that UQH_2 can inhibit lipid peroxidation directly, without the mediation of vitamin E.

Ubiquinone; Coenzyme Q; Vitamin E; Lipid peroxidation; Submitochondrial particle

1. INTRODUCTION

Over the last three decades evidence has accumulated which strongly suggests that coenzyme Q (ubiquinone; UQ) in its reduced form acts as an antioxidant inhibiting lipid peroxidation in model systems [1–8] and in biological membranes in vitro [2,3,9–15] and in vivo [14,16,17]. Although the precise mechanism of this effect is not yet known, it has been suggested [18,19] that UQH_2 may act by quenching lipid peroxyl ($LOO^{\cdot}$), alkoxyl ($LO^{\cdot}$) or carbon based lipid radicals ($L^{\cdot}$), or lipid peroxidation initiating species such as the perferryl radical (Fe^{3+}-$O_2^{-\cdot}$). Alternatively, it has recently been proposed [20] that UQH_2 inhibits lipid peroxidation merely in an indirect fashion, by maintaining vitamin E (α-tocopherol), in its reduced form, i.e. by regenerating vitamin E from the α-tocopheroxyl radical ($E^{\cdot}$) formed during its antioxidant function, which otherwise is commonly attributed to water-soluble reducing agents such as ascorbate or glutathione (see [21] for review).

In the present work we have tested this hypothesis by measuring ascorbate- and ADP-Fe^{3+}-induced lipid peroxidation in beef heart submitochondrial particles (SMP) before and after extraction of endogenous coenzyme Q_{10} (UQ-10) and vitamin E, and after reincorporation of the same components alone or in combination into the SMP. The results show that reduced UQ-10 (ubiquinol, UQH_2) can inhibit lipid peroxidation in the absence of vitamin E, and, thus, that its antioxidant effect cannot be due exclusively to the maintenance of vitamin E in the reduced state. The possible site(s) of action of UQH_2 in relation to that of vitamin E will be discussed.

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2. MATERIALS AND METHODS

The experiments were performed with submitochondrial particles (SMP) prepared from beef heart mitochondria by sonication in the presence of EDTA according to Lee and Ernster [22]. The particles were washed twice with 0.25 M sucrose, once with 0.15 M KCl and then suspended in 0.15 M KCl (20 mg protein/ml). After removing an aliquot for testing the 'native' SMP, the rest of the suspension was lyophilized overnight. Extraction of the lyophilized SMP and reincorporation of UQ-10 were done with pentane as described by Ernster et al. [23], with the following minor modifications of the reincorporation procedure. The extracted SMP (20 mg protein) were suspended in 2 ml pentane containing either 1 mM UQ-10 (Sigma Chemical Co., St. Louis, USA) or 0.75 mM vitamin E (DL-α-tocopherol, Merck Biochemica, Darmstadt, Germany), or 1 mM UQ-10 + 0.75 mM vitamin E, and shaken in an ice-bath for 5 min.

The contents of UQ-10 and vitamin E in the pentane extracts were analyzed by 'reversed-phase' HPLC according to Elmberger et al. [24], after elution with a linear gradient from 90% of methanol/water (9:1) to 100% methanol/isopropanol (4:1) for 30 min, while monitored continuously at 292 nm. Identification was generally based on the commercial samples as standards.

Lipid peroxidation was determined by following O_2 consumption polarographically and by measuring the formation of thiobarbituric acid reactive substances (mainly malondialdehyde and referred to below as MDA) as described by Ernster and Nordenbrand [25].

All incubations were done at 30°C in a 1 ml incubation medium consisting of 25 mM Tris-Cl, pH 7.4, 0.15 M KCl, and SMP containing 0.3–0.5 mg protein. The reaction was started by the addition of 0.25 mM Na-ascorbate followed by 1 mM ADP + 0.01 mM $FeCl_3$ (ADP-Fe^{3+}). When indicated, 2 μM antimycin and 5 mM succinate were added prior to ascorbate and ADP-Fe^{3+}. Lipid peroxidation was terminated by the addition of 0.1% butylated hydroxytoluene. An aliquot of 0.8 ml was removed and mixed with 27 μl 100%

trichloroacetic acid for determination of MDA. Protein was determined by the biuret method [26]. All chemicals used were commercial products.

3. RESULTS

As reported earlier [23], removal of UQ-10 resulted in a virtually complete loss of both NADH and succinate oxidase activities. Respiration with both substrates was reactivated when UQ-10 was reincorporated into the SMP (Table I). Incorporation of vitamin E into the pentane-extracted SMP (see below) did not stimulate respiration either in the absence or presence of UQ-10.

Fig. 1 shows the lipid peroxidation induced by ascorbate and ADP-Fe^{3+} in SMP before (Fig. 1a,b) and after (Fig. 1c,d) lyophilization, and after extraction (Fig. 1e,f) and reincorporation (Fig. 1g,h) of UQ-10. SMP before and after lyophilization exhibited an O_2 consumption with a concomitant MDA formation (Fig. 1a,c). Addition of antimycin and succinate before ascorbate and ADP-Fe^{3+} inhibited both O_2 consumption and MDA formation (Fig. 1b,d). The inhibition of O_2 consumption was only partial; this residual activity was not affected by increasing amounts of antimycin but was inhibited by 1 mM KCN (data not shown). However, MDA formation was inhibited by 95%. A similar inhibition of MDA formation was obtained with antimycin and NADH (0.5 mM), but not with rotenone and NADH (data not shown). Malonate (5 mM) abolished the effect of succinate.

After extraction of UQ-10, neither the O_2 consumption nor the formation of MDA was inhibited by antimycin and succinate (Fig. 1e,f). The inhibition reappeared, as expected, after reincorporation of UQ-10 (Fig. 1g,h), due to the antioxidant effect of UQH_2, in accordance with earlier reports [9,10]. The extracted SMP showed a slight inhibition of MDA formation (see Fig. 1f). This could be due to a small amount of protein-bound UQ-10 that cannot be extracted with the method used [27]. This residual UQ-10 might also explain the low oxidase activity still exhibited by these particles (see Table I).

It may also be seen in Fig. 1 that there was an induction period of O_2 consumption in the native (Fig. 1a) and the lyophilized (Fig. 1c) SMP, but not in the SMP after pentane extraction (Fig. 1e) and reincorporation of UQ-10 (Fig. 1g). Such an induction period has earlier been described with microsomes [28,29] and liposomes [30], and attributed to the presence of vitamin E. It therefore appeared possible that the pentane extraction in our experiments had removed both UQ-10 and vitamin A. As shown in Fig. 2, this indeed was the case; the extracted SMP contained no detectable amounts of UQ-10 and vitamin E as revealed by HPLC analysis of pentane extracts obtained by four successive extractions of the lyophilized SMP.

When vitamin E was incorporated into the extracted SMP, the induction phenomenom reappeared (Fig. 1i). In this particles, MDA formation was only partially inhibited by antimycin + succinate (Fig. 1j), presumably due to a reduction of the α-tocopheroxyl radical through the electron transport system [31], probably via residual UQ-10.

Fig. 3 shows HPLC data on the incorporation of UQ-10 and vitamin E into the extracted SMP alone and in combination. UQ-10 was incorporated into the SMP to a level similar to the original content (Fig. 3b,c), in accordance with earlier reports [23,32]. This level was virtually independent of whether or not vitamin E also was incorporated. The extent of incorporation of vitamin E was dependent on the amount of the vitamin used. With 0.75 mM vitamin E in the pentane solution used for incorporation, it was approximately the same as in the native SMP (Fig. 3b,d); at this level of vitamin E, the induction period was also approximately equal to that found with the native SMP (see Fig. 1a,i).

When vitamin E was combined with UQ-10 in the reincorporation system (Fig. 3e), the amount of vitamin E recovered in the SMP was slightly increased. In this case, the induction period for the onset of O_2 consumption was even longer than with vitamin E alone (data not shown). This relationship will be elaborated on further in a forthcoming paper.

Table I

NADH and succinate oxidase activities of beef heart submitochondrial particles (SMP) before (native) and after lyophilization (lyoph.) and after pentane extraction (lyoph.,extr.) and after reincorporation of coenzyme Q_{10} (UQ-10) and/or vitamin E (Vit. E)

SMP	nanoatoms O/min/mg protein	
	NADH oxidase	Succinate oxidase
Native	1371	392
Lyoph.	796	222
Lyoph., extr.	6	12
Lyoph., extr., UQ-10	380	221
Lyoph., extr., Vit. E	8	16
Lyoph., extr., UQ-10 + Vit. E	438	135

SMP (0.4 mg protein) were incubated in 1 ml medium containing 25 mM Tris-Cl, pH 7.4, 0.15 M KCl, 35 μM cytochrome *c*, and when indicated, 0.96 mM NADH or 5 mM succinate. Temp. 30°C. Lyophilization, pentane extraction, and incorporation of UQ-10 and/or Vit. E were done as described in Materials and Methods.

4. DISCUSSION

The present results confirm earlier studies showing that reduced UQ-10 (ubiquinol, UQH_2) is a potent inhibitor of lipid peroxidation of SMP [9,10]. In addition, the data presented here show that the method used for the extraction of UQ-10 from the SMP also removes vitamin E and, thus, that the presence of vitamin E is not a prerequisite for the function of UQH_2 as an antioxidant, as postulated by Kagan et al. [20] mainly on the basis of experiments with non-extracted membrane

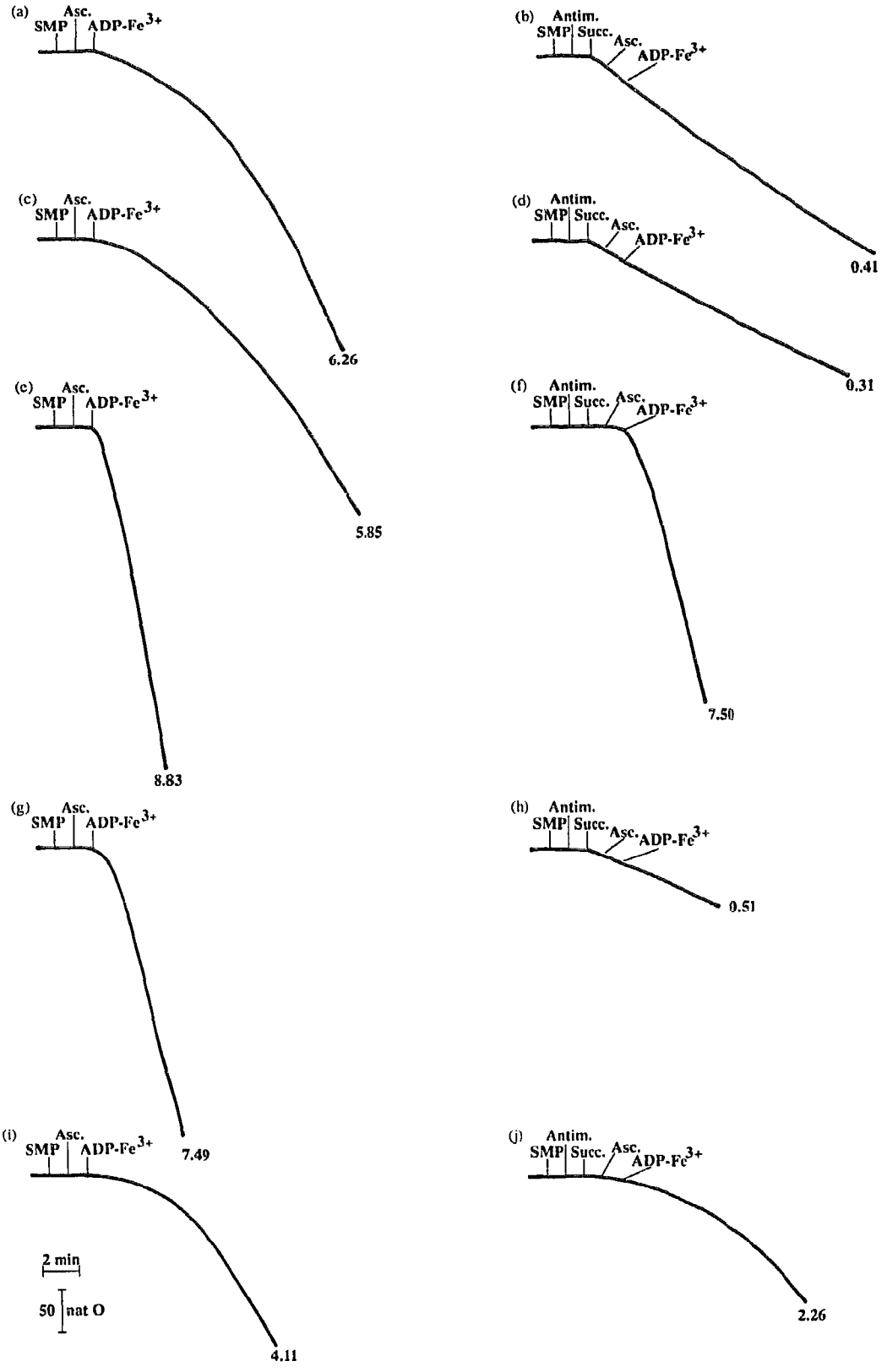


Fig. 1. Ascorbate and ADP-Fe^{3+}-induced lipid peroxidation in SMP before (a,b) and after lyophilization (c,d) and after pentane extraction (e,f) and reincorporation of UQ-10 (g,h) and Vit. E (i,j). The lines represent polarographic traces of oxygen consumption, and the numbers at the end of the traces indicate the amount of MDA formed in nanomoles. The reaction mixtures were as described in Materials and Methods. Abbreviations: SMP, submitochondrial particles; Asc., Na-ascorbate; Antim., antimycin; Succ., succinate.

preparations treated with externally added coenzyme Q. This direct effect of UQH_2 on lipid peroxidation may consist of a quenching of the L· and/or the LOO· radical, as schematically illustrated in Fig. 4. Such an effect of UQH_2 has earlier been demonstrated in model systems [1–8]. Our results do not exclude the possibility that, in addition to its direct effect, UQH_2 may also inhibit lipid peroxidation by maintaining vitamin E in the reduced state, as proposed by Maguire et al. [31] and Mukai et al. [33] which, in turn, quenches LOO· [34].

UQ-10 in mitochondria may be in a particularly favourable position in fulfilling these functions, due to its location in the hydrophobic domain of the membrane, where the initiation of lipid peroxidation takes place and, moreover, to its ready access to an efficient enzymic reduction device in the form of the 'Q cycle',

Fig. 2. HPLC chromatogram of non-pooled pentane extracts from lyophilized SMP obtained by four successive extractions (1–4). UQ-10 and Vit. E were identified on the basis of retention times as obtained with commercial standards. For further details, see Materials and Methods.

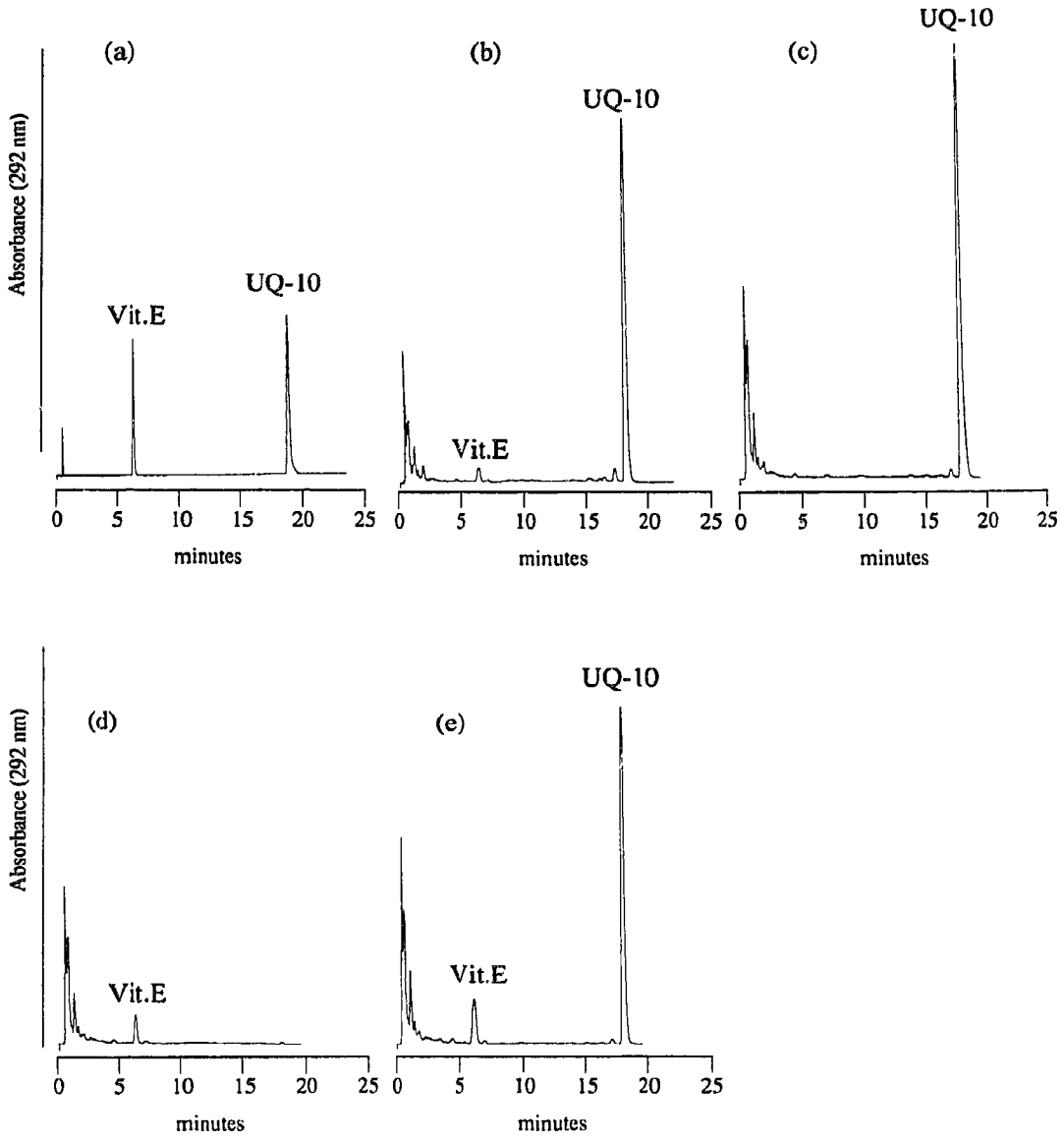
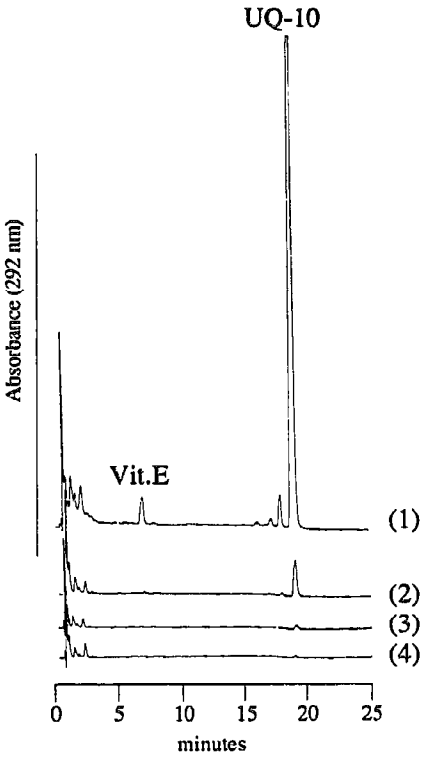


Fig. 3. Incorporation of UQ-10 and/or Vit. E into lyophilized, pentane-extracted SMP. (a) Standards. (b) Three pooled pentane extracts from lyophilized SMP. (c) Three pooled pentane extracts from lyophilized SMP after reincorporation of UQ-10. (d) Three pooled pentane extracts from lyophilized SMP after reincorporation of Vit. E. (e) Three pooled pentane extracts from lyophilized SMP after reincorporation of UQ-10 and Vit. E.



which can regenerate UQH_2 from UQ-10-semiquinone ($UQ^{-\cdot}$) [35]. The relationship between UQH_2 and vitamin E in inhibiting lipid peroxidation in biological membranes other than mitochondria will require further investigation.

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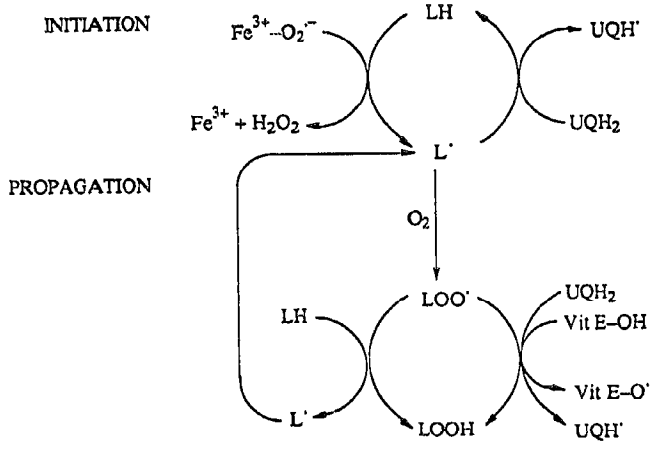

Fig. 4. Proposed sites of action of reduced UQ-10 (UQH_2) and vitamin E (Vit E-OH) as inhibitors of lipid peroxidation. For explanation, see text.

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