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Ubiquinone is reduced by lipoamide dehydrogenase and this reaction is potently stimulated by zinc

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Abstract Ubiquinol is an endogenously synthesized lipid-soluble antioxidant. Regeneration of ubiquinol from the oxidized form is essential to the maintenance of its antioxidant function. We demonstrated that lipoamide dehydrogenase can reduce ubiquinone to ubiquinol. Zinc increased the rate of the NADPH-dependent reduction more than 10-fold. The concentration ubiquinone resulting in the half-maximal rate of reduction was approximately 5 μM in the presence and 4 μM in the absence of zinc. These data may explain how ubiquinone is reduced to the active antioxidant ubiquinol, which plays such an important role in protecting against oxidative stress and lipid peroxidation.

Key words: Ubiquinone; Lipoamide dehydrogenase; Antioxidant; Zinc

1. Introduction

Reactive oxygen species (ROS) can cause oxidative damage to living cells due to their high level of reactivity. The polyunsaturated fatty acids in biological membranes are particularly sensitive to oxidative damage by ROS. Reaction of these species with unsaturated fatty acids initiates a chain-reaction leading to extensive damage to the membrane, a process known as lipid peroxidation [1]. This process can be prevented by lipid-soluble antioxidants such as vitamin E (α-tocopherol) and ubiquinol [1], which function as antioxidants only in the reduced state. Therefore, when tocopherol and ubiquinol are oxidized by ROS their reduced forms must be regenerated.

Ubiquinone (Q10) is an obligatory participant in the mitochondrial respiratory chain, where it is present in large excess over the other components involved in electron transport in order to achieve maximal respiration [2]. In recent years ubiquinol (reduced Q10) has been the object of renewed interest, since accumulating evidence indicates that this substance is our only endogenously synthesized lipid-soluble antioxidant and is present in all membranes [1,3–5]. The mechanism by which Q10 is reduced to ubiquinol is not known although different cytosolic enzymes have been proposed to be involved [6,7]. However, no specific enzyme catalyzing this reaction has been identified and therefore, we have investigated the well-known enzyme, lipoamide dehydrogenase (EC 1.8.1.4), in this regard.

Lipoamide dehydrogenase is a component of the 3-α-keto-acid dehydrogenase complex which oxidizes pyruvate, α-ketoglutarate and the branched-chain α-ketoacids. This enzyme complex is present at the matrix surface of the inner mitochondrial membrane in all eukaryotic organisms studied to date. It catalyzes the oxidative decarboxylation of pyruvate with concomitant formation of CO_2, acetyl-CoA and NADH. In this complex (dihydro)lipoamide dehydrogenase catalyzes NAD-dependent oxidation of the dihydrolipoyl cofactor covalently linked to the components catalyzing acetyl transferase.

Lipoamide dehydrogenase belongs to a family of pyridine nucleotide-disulfide oxidoreductases [8], which are homodimeric proteins with subunits approximately 50 kDa in size, each containing a FAD moiety and a redox-active disulfide. Lipoamide dehydrogenase is ubiquitous in aerobic organisms, including prokaryotes, eukaryotes and archaebacteria. There are certain indications that this enzyme is present not only in the mitochondria, but also in plasma membranes ([9], Björnstedt et al., in preparation). Lipoamide dehydrogenase also reduces the low-molecular weight antioxidant thiols lipoamide and lipoic acid [8]. The latter has been proposed as having beneficial effects in the treatment of diseases of the liver [10].

2. Materials and methods

2.1. Chemicals

Methanol; petroleum ether (b.p. 40–60°C); 2-propanol and *n*-hexane of analytical grade; $CuSO_4 \cdot 5H_2O$; Na-K-tartrate·$4H_2O$; NaOH; Na_2CO_3; NaDOC and $ZnCl_2$ were purchased from KEBO (Stockholm, Sweden). NADH, NADPH, EDTA, Trizma base and ubiquinone-6 and -10 were obtained from Sigma (St. Louis, MO, USA).

2.2. Enzyme preparation

Lipoamide dehydrogenase from porcine heart (type III; L2002) was delivered by Sigma as a suspension in 3.2 M ammonium sulfate. This suspension was centrifuged at 10 000 rpm for 5 min in Eppendorf tubes, the supernatant discarded and the pellet dissolved in 50 mM Tris-HCl, pH 7.5. Remaining ammonium sulfate was removed by chromatography on a Sephadex G-25 column (NAP-5; Pharmacia, Sweden). This column was pre-equilibrated with 50 mM Tris-HCl, pH 7.5 and the protein was eluted using the same buffer. Protein was quantified using the procedure of Lowry and coworkers [11] and the solution then diluted to obtain 1 mg protein/ml. The activity of the purified protein was measured as the oxidation of NADH (spectrophotometrically at 340 nm) in the presence of lipoamide.

2.3. In vitro incubation with ubiquinone

The 100 μl assay mixtures contained 50 mM Tris-HCl, pH 7.5, 1.0 mM EDTA, 0.1% Triton X-100, NADPH or NADH (1.6 mg/ml) and up to 50 μM ubiquinone-10. Ubiquinone was dissolved in ethanol, but the final content of ethanol in the mixtures did not exceed 2% in any incubation. In some experiments $ZnCl_2$ was added to give a final concentration of 500 μM and in these experiments EDTA was absent. The reaction was started by the addition of lipoamide dehydrogenase in different amounts up to 30 μg. After the addition of all components, the test tubes were flushed with nitrogen,

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Abbreviations: LipDH, lipoamide dehydrogenase; Q10, ubiquinone-10; ROS, reactive oxygen species

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covered, vortexed and placed in a water bath at 37°C for incubation for the different time periods indicated. Reactions were terminated by the addition of methanol.

2.4. Extraction procedure

Lipid extraction was performed according to a procedure described previously [5], with certain modifications. After incubation, 5 ml methanol was added followed by 400 μl distilled water and 0.68 nmol Q6 (as internal standard). Subsequently 3 ml petroleum ether (b.p. 40–60°C) was used to extract ubiquinone and ubiquinol. To accomplish this the liquid phases were mixed by shaking and the test-tubes then centrifuged at 1000 rpm for 3 min at room temperature. The upper petroleum ether phase was removed and evaporated under nitrogen. The residue was dissolved in 25 μl methanol:*n*-hexane:2-propanol, 2:1:1, and injected directly onto the HPLC column. This entire procedure was completed within 10 min.

2.5. HPLC analysis

Ubiquinone and ubiquinol were quantitated by reversed-phase HPLC (LC-6A, Shimadzu, Kyoto, Japan) using a Hewlett Packard Hypersil ODS 3 μm column. The solvents employed were: in pump system A, methanol:water, 9:1 and in system B, methanol:2-propanol:*n*-hexane, 2:1:1. Linear gradients from 10–50% solvent B were run for 15 min, followed by 50–55% solvent B for 8 min, and, finally, 55–100% solvent B for an additional 3 min, all at a flow rate of 1.5 ml/min. The temperature was maintained at 15°C. Ubiquinone-6, ubiquinol-10 and ubiquinone-10 were monitored with an UV-detector (Shimadzu, L-ECD-6A), and eluted in three well-separated peaks with retention times of 10–11, 19–20 and 25–26 min, respectively.

3. Results

The addition of ubiquinone to an incubation mixture containing lipoamide dehydrogenase and NADH or NADPH resulted in the reduction of this compound to ubiquinol. With 100 pmol enzyme, approximately 23 and 19 pmol of ubiquinone, respectively, was reduced per min. The rate of this reaction was dependent on the amount of lipoamide dehydrogenase present (Fig. 1) and the reaction showed an exponential configuration.

Zinc is known to inhibit several members of the pyridine nucleotide-disulfide oxidoreductase family. Thus, the influence of this cation on the reduction of ubiquinone by lipoamide dehydrogenase, was examined. In this system the rate of the NADPH-dependent reaction was increased more than 10-fold (Fig. 2), whereas the rate of the NADH-dependent reaction was not significantly altered by zinc. Other divalent cations (Ca^{2+}, Mg^{2+}, Mn^{2+}) did not potentiate the rate of the reaction (Xia et al., in preparation).

The concentration of ubiquinone resulting in half-maximal rate of reduction (apparent K_m) was approximately 5 μM with and 4 μM without zinc (Fig. 3). Since the reaction rate increase exponentially with increasing enzyme concentration, as shown in Fig. 1, the turnover number (K_{cat}) has to be given at

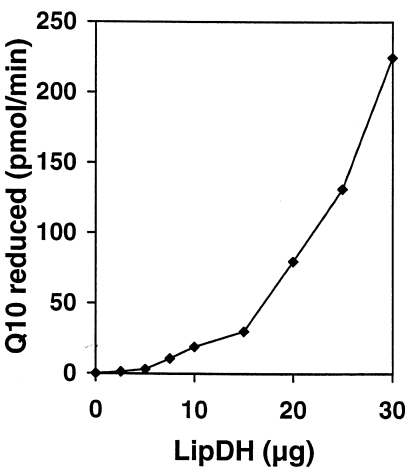

Fig. 1. Reduction of ubiquinone by different amounts of lipoamide dehydrogenase (LipDH) using NADPH as co-factor. Enzyme (0–30 μg) was added to 50 mM Tris-Cl, pH 7.5, containing Triton X-100, NADPH and ubiqiunone (Q10). Ubiquinone and ubiquinol were extracted and analyzed by HPLC as described in Section 2. The values given are the means of three different experiments at each enzyme concentration indicated.

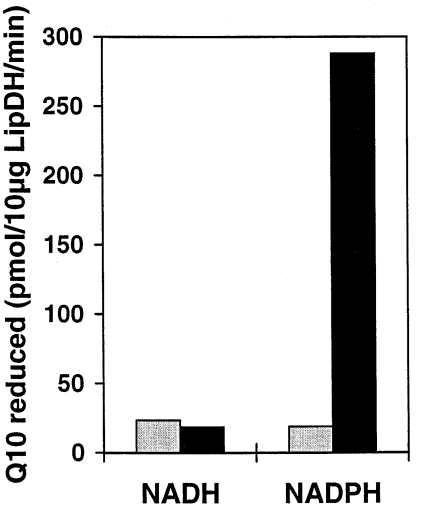

Fig. 2. Zinc-dependent potentiation of the reduction of ubiquinone by lipoamide dehydrogenase (LipDH). Lipoamide dehydrogenase (10 μg), ubiquinone, NADPH and Triton X-100, were incubated for 20 min in the absence (light bars) or presence (dark bars) of zinc. Ubiquinone and ubiquinol were extracted and analyzed by HPLC as described in Section 2.

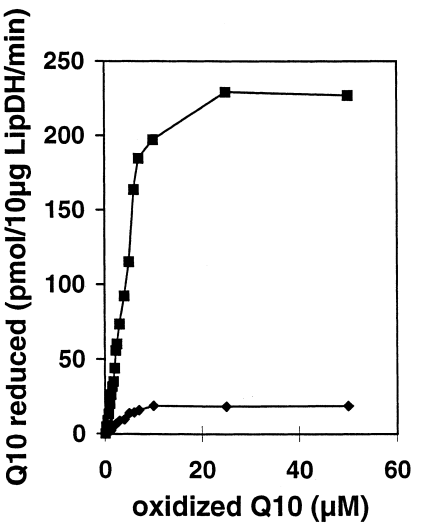

Fig. 3. Dependence of the reduction of ubiquinone (Q10) by lipoamide dehydrogenase on substrate concentration in the absence and presence of zinc. The reactions were performed as described in the legend to Fig. 1 except that increasing concentrations of ubiquinone were employed. The concentration of ubiquinone giving the half-maximal rate was approximately 5 μM with zinc (■) and 4 μM without zinc (◆) and the half-maximal rates were 115 and 10 pmol Q10 reduced/min, respectively. The values given are the means of three different experiments.

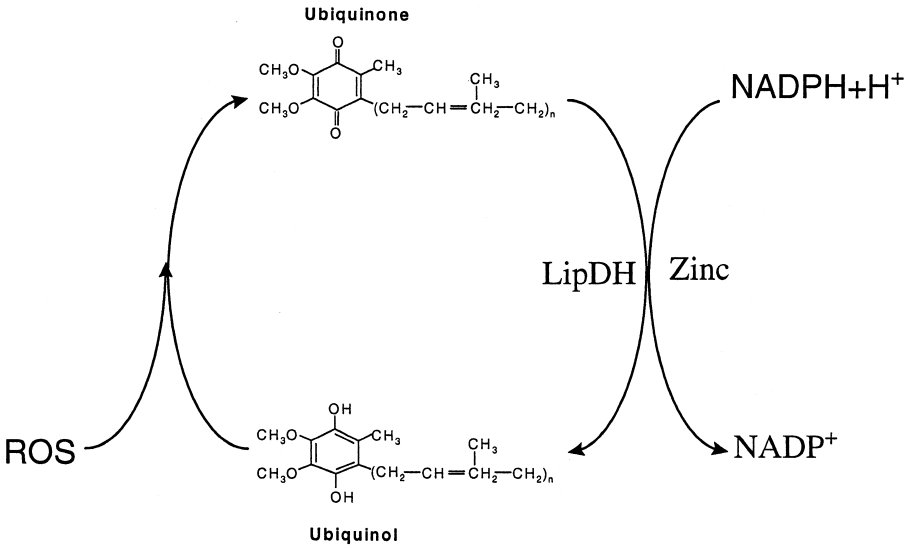

Fig. 4. Schematic illustration of the reduction of ubiquinone by lipoamide dehydrogenase (LipDH) and the scavenging of ROS by ubiquinol.

a specified enzyme concentration. At the enzyme concentration used in these experiments (1 μM) the K_{cat} was approximately 2.4 min^{-1} in the presence and 0.2 min^{-1} in the absence of zinc.

4. Discussion

This investigation clearly demonstrates that ubiquinone is reduced to its active antioxidant form ubiquinol by lipoamide dehydrogenase with either NADH or NADPH as the cofactor. Furthermore, this reaction is potently stimulated by zinc, but only when NADPH is the co-factor. This is especially interesting in light of the fact that NAD(H) is the co-factor generally employed in the extensive studies on lipoamide dehydrogenase in mitochondria.

Redox reactions of (dihydro)lipoamide involves the sulfhydryl groups in the active site of lipoamide dehydrogenase. However, these reactions are efficiently inhibited by zinc (Xia et al., in preparation). The potent stimulation of the reduction of ubiquinone by zinc suggests that different active sites on the enzyme molecule may be involved in the two different reactions. The site responsible for the reduction of ubiquinone most likely contains the FAD moiety. The potent effect of zinc may involve a conformational change in lipoamide dehydrogenase and is now under further investigation at our laboratory.

Located in the immediate vicinity of ubiquinone in the mitochondria, lipoamide dehydrogenase is part of the pyruvate dehydrogenase complex. This enzyme has also been detected in the plasma membrane ([9], Björnstedt et al., in preparation). These localizations of ubiquinone and lipoamide dehydrogenase as well as the fact that ubiquinone is taken up by cells [12], strongly support the physiological relevance of our observations.

In cells constantly exposed to ROS, antioxidants play an important role in preventing damage to biological membranes from lipid peroxidation. Reduction of ubiquinone by lipoamide dehydrogenase and the strong potentiation of this reaction by zinc may explain how levels of ubiquinol are restored, forming an efficient pathway (Fig. 4) for the elimination of ROS.

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