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I. INTRODUCTION

Coenzyme Q (CoQ) or ubiquinone is distributed ubiquitously in the natural kingdoms. CoQ homologs (CoQ_n) containing 9 and 10 isoprene units occur in mammals, which coexist in different amounts depending on the species. CoQ_9 is the predominant form in mouse and rat, and CoQ_{10} is predominant in rabbit, guinea-pig, dog, pig and man [1-3].

CoQ plays an important role in ATP synthesis as an electron-carrying component of the mitochondrial respiratory chain. In recent years, increasing attention has focused on CoQ_n as an antioxidant both *in vitro* and *in vivo*, and the antioxidant activity of CoQ_n is exhibited by its reduced form, CoQ_nH_2 [4,5]. There are some excellent recent reviews on the function of CoQ_nH_2 in this area [4-6].

In view of all the facts reported relatively little is known about the mechanism of the antioxidant function of CoQ_nH_2 at cellular and biomembrane level. It is not yet known whether CoQ_9H_2 and $\text{CoQ}_{10}\text{H}_2$ coexisting in the cell play the same or different antioxidative roles and further, whether CoQ_nH_2 acts alone or in concert with other cellular antioxidants, such as α -tocopherol.

To solve these problems, we have carried out a series of *in vivo* experiments [7-11], in which antioxidative action of endogenous CoQ_nH_2 was determined, together with the protective effect of CoQ_{10} accumulated and reduced to $\text{CoQ}_{10}\text{H}_2$ in the tissue after injection on peroxidative reperfusion injury.

Then, we have investigated antiperoxidative activity of endogenous CoQ_9H_2 and $\text{CoQ}_{10}\text{H}_2$ in rat and guinea-pig hepatocytes exposed to a water-soluble radical initiator, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The results obtained indicate a difference in the antioxidant activity between CoQ_9H_2 and $\text{CoQ}_{10}\text{H}_2$, together with the action of CoQ_nH_2 independent of α -tocopherol.

II. EVIDENCE FOR *in vivo* ANTIOXIDATIVE ACTIVITY OF REDUCED CoQ

Good evidence for *in vivo* antiperoxidative activity of CoQ_nH_2 was obtained from studies with experimental endotoxemia in mice [7,8]. Endotoxin-induced lipid peroxidation in the liver was found to be associated with drastic decreases in hepatic levels of CoQ_9H_2 , α -tocopherol, and reduced glutathione (GSH). Administered CoQ_{10} (oxidized form), after accumulation in the liver and reduction to $\text{CoQ}_{10}\text{H}_2$, prevented the decreases in endogenous antioxidants, such as CoQ_9H_2 , α -tocopherol and GSH, completely suppressed lipid peroxidation, and markedly increased the survival rates of endotoxemic mice.

Other findings indicating *in vivo* antioxidant activity of CoQ_nH_2 include decreased tissue levels of CoQ_9H_2 , $\text{CoQ}_{10}\text{H}_2$, α -tocopherol and GSH, which was accompanied by the increases in tissue and mitochondrial lipid peroxides in rat liver following ischemia and reoxygenation [9,10]. Administered CoQ_{10} has been shown to prevent the decreases in these endogenous antioxidants after reduction

to CoQ₁₀H₂ in the liver, and to protect against acute postischemic hepatic injury in a similar mechanism to that found in the experimental endotoxemia.

In another model experiment on canine heart preservation and the following reperfusion, the antioxidative activity of administered CoQ₁₀, was also demonstrated [11]. The accumulated CoQ₁₀H₂ protected the organ against reperfusion injury and maintained left ventricular functions to almost the normal level by suppressing the stimulated lipid peroxidation and by preventing the decreases in endogenous CoQ₉H₂ and α -tocopherol.

III. ANTIOXIDANT ACTIVITY OF CoQ HOMOLOGS IN RADICAL-INDUCED HEPATOCYTE INJURY

As described above, CoQ₉H₂ and CoQ₁₀H₂ coexisting in animal cells, especially hepatocytes, are not known whether they have the same antioxidant activity or not. An approach to studying possible differences in the antioxidant role of CoQ homologs in animal cells is to know differences in subcellular distributions. Subcellular fractionation of rabbit liver revealed that most CoQ₉ existed in the cytosolic fraction, and most CoQ₁₀ existed in the mitochondrial fraction [3]. In addition, in rabbit liver %CoQ₉H₂ in total (oxidized plus reduced) CoQ₉ increased with growth at a rate much slower than that of %CoQ₁₀H₂, since CoQ₁₀ in mitochondria is expected to be more easily reduced by the respiratory chain than CoQ₉ in cytosol. These results suggest possible differences in the role of CoQ₉ and CoQ₁₀ in mammalian cells, together with the mechanism of antioxidant activity of CoQ homologs.

A water-soluble radical initiator, AAPH, induces peroxidative cellular damage from the outside of the cell [12, 13]. The concentration of CoQ₉H₂ in rat hepatocytes containing total CoQ₉ and CoQ₁₀ at a ratio of 6:1 decreased linearly after the addition of AAPH with a reciprocal increase in CoQ₉ (oxidized form). Although CoQ₁₀H₂ tended to decrease slightly during the incubation with AAPH, the change was not significant. No loss of cell viability or an increase in lipid peroxidation was observed until most of CoQ₉H₂ was consumed [14].

In guinea-pig hepatocytes containing total CoQ₉ and CoQ₁₀ at a ratio of 1:5, the consumption of CoQ₁₀H₂ was accompanied by an increase of TBARS when incubated with AAPH, indicating the effective antioxidant activity of CoQ₁₀H₂ in these cells. At the same time, complete consumption of cellular CoQ₉H₂ was also observed. The viability of AAPH-treated guinea-pig hepatocytes remained comparable to that of the control until consumption of CoQ₁₀H₂ and CoQ₉H₂ reduced their levels to about 20% of the original. If the insignificant consumption of CoQ₁₀H₂ in AAPH-treated rat hepatocytes is compared with the rapid consumption of CoQ₉H₂ in the guinea-pig cells, CoQ₉H₂ appears to be a potent antioxidant, with activity independent of its concentration [14].

IV. ANTIOXIDANT ACTIVITY OF REDUCED COENZYME Q HOMOLOGS IN RELATION TO THAT OF α -TOCOPHEROL

α -Tocopherol is a good chain breaking antioxidant in biomembranes. Recent reports have proposed some explanations for the mechanism of CoQ_nH₂ antioxidant activity in relation to the action of α -tocopherol; (a) CoQ_nH₂ directly scavenges lipid peroxyl radicals as effective as α -tocopherol [15], and (b) CoQ_nH₂ acts through recycling of α -tocopherol [16,17].

In hepatocytes treated with AAPH, α -tocopherol was consumed with time at the rate comparable to that of either CoQ₉H₂ in rat hepatocytes or CoQ₁₀H₂ and CoQ₉H₂ in guinea-pig hepatocytes [14] as shown in Fig. 1. This suggests that

both antioxidants act independently in the hepatocytes when AAPH induces cellular injury.

Fig. 1. Effect of AAPH on the concentrations of endogenous CoQ_9H_2 , and $\text{CoQ}_{10}\text{H}_2$ and α -tocopherol in rat and guinea-pig hepatocytes. The values are expressed as percentage of the concentrations obtained at 0-time incubation [14].

SUMMARY

Evidence for *in vivo* antioxidative activity of reduced CoQ homologs has been presented. This came from studies with experimental endotoxemia in mice, reoxygenation of rat liver following ischemia, and reoxygenation of canine heart following 24-hour cold preservation. In radical-induced injury of hepatocytes, it has been first shown that reduced CoQ_9 acts as a potential antioxidant regardless of its cellular concentration, whereas reduced CoQ_{10} acts in cells containing CoQ_{10} as the predominant homolog. The antioxidant activity of reduced CoQ homologs appears to be independent of that of α -tocopherol under the conditions employed.

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