Short Communication

Biosynthesis of Ubiquinone in Rat Liver*

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Ubiquinone has a broad distribution in higher organisms, being present in all organs and tissues of all animals investigated to date. During the biosynthesis of ubiquinone, the polyisoprenoid portion of the molecule solanesol pyrophosphate is transferred to 4-hydroxybenzoate and in a series of reactions, the ring is subsequently substituted at carbons 2, 3 and 5.[1] The isoprenoid moiety differs in two respects from that of dolichol, the other major cellular polyisoprenoid: in ubiquinone, the isoprene residues are all *trans,* while in dolichol they are predominantly *cis*. Whereas all species possess a family of dolichols, only one major form of ubiquinone is found in each animal, e.g. 9 isoprene units in length in the rat and 10 isoprene units in length in man.

Ubiquinone is present in high concentration in the inner mitochondrial membrane. Its main established role is participation in the respiratory chain and associated oxidative phosphorylation as an ubiquinone/ubiquinol redox couple.[2] It was recently established that polyisoprenoids of the dolichol type are synthesized at the endoplasmic reticulum by the same initial biosynthetic steps that lead to cholesterol.[3] Consequently, it seemed reasonable to investigate the possibility that either the isoprenoid portion of ubiquinone or the whole molecule is also synthesized in microsomes.

Ubiquinone was found to be present in all cellular membranes in relatively high amounts, the levels being equally high in Golgi vesicles, lysosomes and mitochondria (Table 1). The concentration of this lipid in microsomes was low, while peroxisomes and plasma membranes demonstrated two- and five-fold higher levels, respectively. Analysis using marker enzymes demonstrated that the presence of ubiquinone in microsomes was not the result of contamination by other cellular organelles. The other fractions prepared were also cross-contaminated to a limited extent only, as shown previously.[4]

Ubiquinone biosynthesis was followed by incubation of slices with two different precursors: [^{3}H]-tyrosine, which is utilized for the synthesis of 4-hydroxybenzoate and [^{3}H]-mevalonate, precursor of the isoprenoid unit. After 10 min incubation with [^{3}H]-tyrosine, extensive labelling of microsomal ubiquinone was obtained, in contrast to the mitochondrial compound, which was hardly labelled at all (Fig. 1A). During subse-

Table 1. Subcellular distribution of ubiquinone in rat hepatocytes.

Fractions	Ubiquinone-9 (μg/mg protein)
Homogenate	0.79
Golgi vesicles	2.62
Lysosomes	1.86
Mitochondria	1.40
Inner mitochondrial membranes	1.86
Microsomes	0.15
Peroxisomes	0.29
Plasma membranes	0.74
Supernatant	0.02

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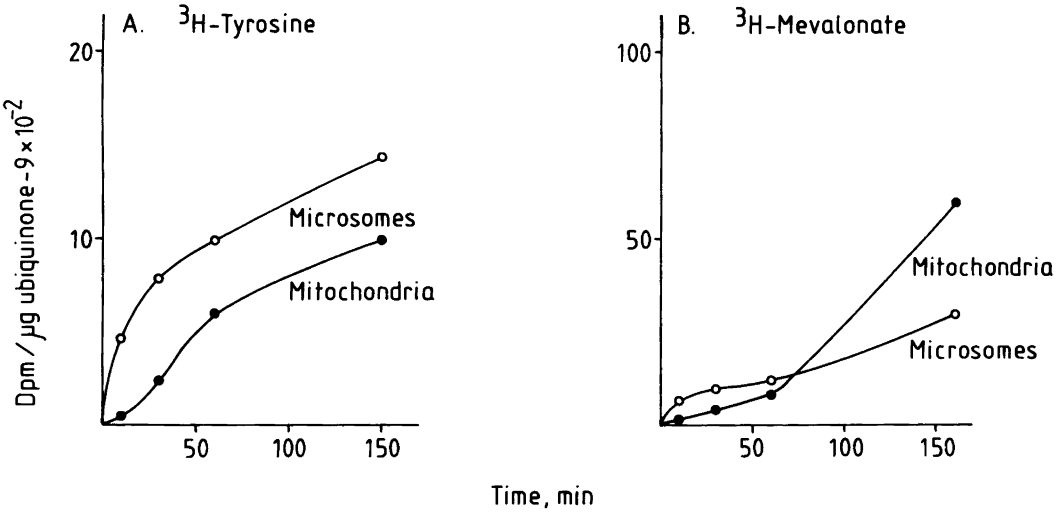

Fig. 1. Incorporation of radioactive precursors into mitochondrial and microsomal ubiquinone.

quent hours, the specific radioactivity in mitochondrial ubiquinone increased greatly but labelling in the microsomes remained higher. [^{3}H]-Mevalonate was recovered extensively in cholesterol and dolichol even in the initial phase of incubation, but this was not the case for ubiquinone (Fig. 1B). During the first 60 min, the specific radioactivity in this latter lipid was relatively low and significantly higher in microsomes than in mitochondria. After 1 additional h, the extent of labelling in mitochondria was twice as high as in microsomes.

Our measurements revealed that rat serum contains 0.15 µg ubiquinone per ml. If the newly synthesized lipid appears in the blood shortly after *in vivo* labelling, it probably originates from the endoplasmic reticulum of the hepatocytes, being secreted by the liver like the blood lipoproteins.[5] In fact, when [^{3}H]-mevalonate was injected into the portal vein, rapid labelling of ubiquinone in the serum was observed, exceeding the specific radioactivity of ubiquinone recovered in the microsomes (Fig. 2). This pattern of blood ubiquinone labelling is very similar to that observed for newly secreted proteins and lipids synthesized in the liver endoplasmic membrane system.

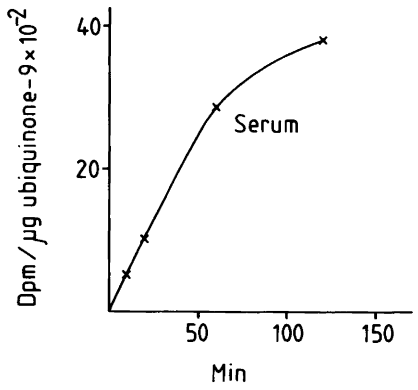

Fig. 2. In vivo labelling of ubiquinone in rat serum.

Ubiquinone is present not only in the inner mitochondrial membrane, but in all organelles of the hepatocytes. Cholesterol and dolichol are synthesized to a large extent at the endoplasmic reticulum and it therefore seems reasonable that at least the polyisoprenoid portion of ubiquinone is also synthesized by this organelle. This site of synthesis is suggested by the results of mevalonate labelling. In addition, the high rate of [^{3}H]-tyrosine incorporation indicates that not only the isoprenoid side chain, but also the entire ubiquinone molecule can be synthesized at the endoplasmic reticulum. It appears, therefore, that this redox lipid is synthesized both in mitochondria and in the endoplasmic reticulum. Quantitatively, the amount produced by the endoplasmic

reticulum may be considerably less than that produced in mitochondria; however, under certain conditions, such as mitochondrial damage or induction of the microsomal system by drugs, synthesis of ubiquinone at the endoplasmic reticulum may be of increased importance. In addition, synthesis at this site may have a specific function(s), e.g., the formation of ubiquinone destined for the blood. It will be a future task to establish to what extent the biosynthesis of ubiquinone in mitochondria and the endoplasmic reticulum follow the same or different pathways.

Experimental

Liver slices with a thickness of 0.5 mm were prepared using a Stadie-Riggs microtome. These slices were incubated either with 250 μCi [^{3}H]-tyrosine (80 Ci mmol^{-1}) or 250 μCi [^{3}H]-mevalonate (13.8 Ci mmol^{-1}) in a total volume of 3 ml of Krebs-Henseleit buffer at 37 °C. Subsequently, mitochondria and microsomes were prepared and used for extraction of lipids. To investigate labelling of ubiquinone in rat serum *in vivo,* [^{3}H]-mevalonate (200 μCi/100 g body weight) was injected into the portal vein of rats. At various times, the animals were decapitated and blood collected. The lipids from slices and blood were extracted with chloroform/methanol, 2:1 (*v/v*), and passed through a silica Sep-Pak. The flow-through fraction containing neutral lipids was passed through C18 Sep-Pak, which was eluted with methanol. When the slices were incubated with [^{3}H]-mevalonate, ubiquinone was separated by additional TLC on silica using hexane/diethyl ether/acetic acid, 75:25:1 (*v/v*), as the developing medium. The lipid extracted was quantitated by HPLC on a C18 reversed-phase column, where the solution in pump A was methanol/water, 9:1 (*v/v*), and in pump B methanol/isopropanol, 4:1 (*v/v*). Elution was monitored at 275 nm and aliquots of the fractions collected were taken for determination of radioactivity. Ubiquinone-6 was added to the homogenate as an internal standard.

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References

1. Olson, R. E. and Rudney, H. *Vitam. Horm. 40* (1983) 1.
2. Norling, B., Glazek, E., Nelson, B. D. and Ernster, L. *Eur. J. Biochem. 47* (1974) 475.
3. Hemming, F. W. In: Porter, J. W. and Spurgeon, S. L., Eds., *Biosynthesis of Isoprenoid Compounds,* Wiley, New York 1983, Vol. II, p. 305.
4. Bergman, A. and Dallner, G. *Biochim. Biophys. Acta 433* (1976) 496.
5. Palade, G. E. *Science 189* (1975) 347.

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