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Ubiquinone in the Lysosomal Membrane Fraction of Rat Liver

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Summary: Ubiquinone has been identified in the membrane fraction of Triton WR 1339-filled rat liver lysosomes. The isoprenoid side chain has been found to consist of Q_9 with traces of Q_7. The content of total ubiquinone in lysosomal membranes is 9.9 ± 0.5 nmol/mg of lysosomal membrane protein.

Ubichinon in der Lysosomenmembran-Fraktion aus Rattenleber

Zusammenfassung: In der Membranfraktion von Leberlysosomen Triton-WR-1339-behandelter Ratten wurde Ubichinon identifiziert. Die Länge der Isoprenseitenkette wurde mit 9 Isopreneinheiten bestimmt. In Spuren wurde auch Q_7 gefunden. Die quantitative Bestimmung ergab $9{,}9 \pm 0{,}5$ nMol/mg Protein in Lysosomenmembranen.

In previous studies on the chemical composition of the secondary lysosomal membrane[1] we observed an unidentified compound in the neutral lipid fraction. The present qualitative and quantitative analysis identified this compound as ubiquinone, a constant constituent of the lysosomal membrane fraction.

Results and Discussion

Lysosomal membranes can easily be purified by hypoosmotic treatment of purified Triton WR 1339-filled lysosomes (tritosomes) and are obtained in an homogeneous form (Fig. 1). We observed that this membrane fraction contains an unidentified component in the neutral lipid fraction. Fig. 2 represents the thin-layer chromatographic analysis of this fraction. The identity of this compound was established by the following criteria: 1) The UV spectrum shows an absorption maximum at 275 nm in ethanol which disappears upon reduction with $NaBH_4$. Alkali has no effect on the extinction at 275 nm. An isosbestic point is seen at 237 nm. 2) Identical R_F values with synthetic ubiquinone in thin-layer chromatography in two different solvent systems. The spots showed the characteristic quenching under UV light. 3) Determination of the isoprenoid side chain length showed this compound to consist mainly of Q_9 and of traces of Q_7 as calculated according to Wagner and Dengler[2] using Q_{10} as a reference substance.

The table shows a 5.2 times higher content of ubiquinone on a protein basis in lysosomal membranes than in mitochondria. The value in mitochondria agrees well with data reported by other authors[3,4].

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Enzymes:

Cytochrome oxidase, ferrocytochrome *c*:oxygen oxidoreductase (EC 1.9.3.1)

NADH dehydrogenase, NADH:(acceptor) oxidoreductase (EC 1.6.99.3).

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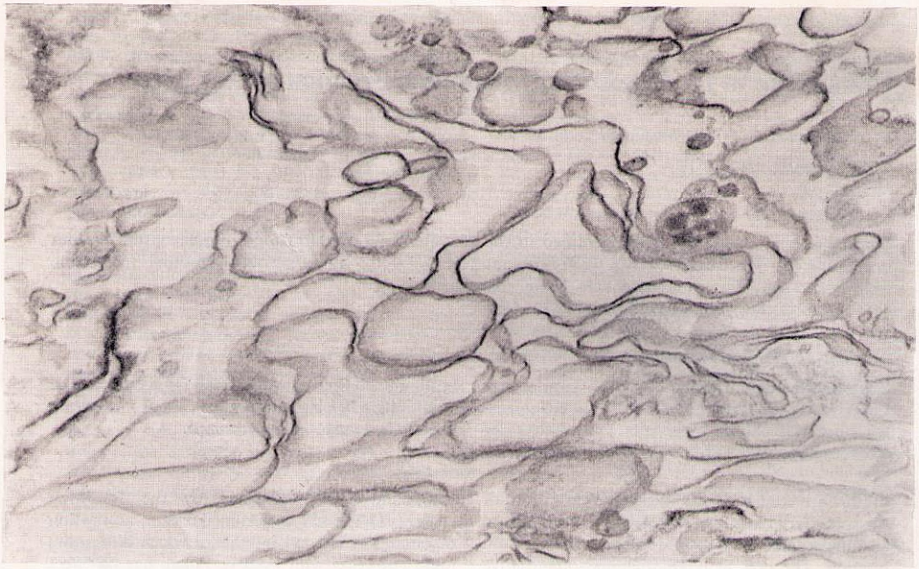

Fig. 1. Electron micrograph of a tritosomal membrane fraction (magnification: x 56000).

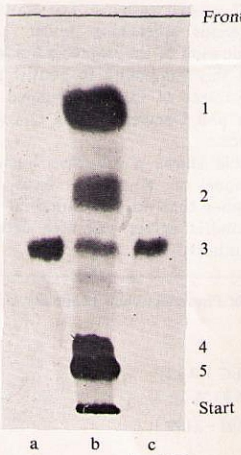

Fig. 2. Thin-layer chromatography of the neutral lipids of the tritosomal membrane fraction. ▶

Table. Ubiquinone content and NADH dehydrogenase activity in subcellular fractions.

Numbers of determinations are given in the brackets.

	Ubiquinone (nmol/mg prot.)	NADH dehydrogenase [U/mg prot.]
Lysosomal membranes	9.9 ± 0.52 (4)	3.0 (2)
Total lysosomes	–	2.5 (2)
Mitochondria („lysosomal free")	1.9 ± 0.3 (4)	68.0 (2)
Cell sap	–	224.0 (2)

Solvent system: petrol ether/ether 70:30. a) Ubiquinone (Q_9) isolated from tritosomal membranes; b) neutral lipids of tritosomal membranes; c) synthetic ubiquinone (Q_{10}); 1) esterified cholesterol; 2) triacylglycerols; 3) ubiquinone; 4) fatty acids + diacylglycerols; 5) cholesterol.

For the elucidation of the biological significance of this high ubiquinone content in the lysosomal membrane fraction the electron transport activity was checked using NADH as substrate and 2,6-dichlorophenolindophenol as electron acceptor. NADH was chosen because Tappel[5] reported that lysosomal preparations cannot oxidize mitochondrial substrates like succinate. As seen in Table 1 the lysosomal dehydrogenase activity is negligible and is regarded as being due to a slight contamination with cell sap protein since the level of mitochondrial contamination lies below 0.1%, as determined by cytochrome *c* oxidase[1].

Since ubiquinone apparently is not used in an electron transfer system, it can be considered a residue of an autophagic uptake of mitochondria by lysosomes. Mitochondrial proteins and most of the lipids are degraded by lysosomal enzymes, whereas aliphatic and aromatic substances cannot be attacked by lysosomes. This agrees well with the data of Tappel[5], who found hemochromes in Triton-filled lysosomes. The spectral properties of these hemochromes were identical with those arising by exposure of isolated mitochondria to lysosomes at 37°C *in vitro*. This experiment provided evidence for the mitochondrial origin of lysosomal hemochromes. Furthermore these hemochromes could not be reduced by mitochondrial substrates. Other mitochondrial constituents such as flavins and heavy metals also have been reported as being present in Triton WR 1339-filled lysosomes[5].

Since ubiquinones are highly enriched in tritosomal membranes, two questions must be raised. Firstly, if ubiquinone is regarded as a real constituent of tritosomal membranes, this would be consistent with the contribution of the Golgi apparatus to the formation of primary lysosomes as discussed by many authors[6,7]. Nyquist *et al.*[8] reported a high content of ubiquinone (3.2 nmol/mg prot.) in the Golgi apparatus with no electron transfer activity in this fraction. Secondly, these compounds could also originate from the residue of undigested material sedimenting together with the tritosomal membranes after the disruption of tritosomes. The presence of ubiquinones would suggest that the electron microscopically homogeneous tritosomal membrane fraction is of heterogeneous origin, namely the true tritosomal membrane and those components derived from residual intracellular membranes autophagized by the lysosomes. This would support the concept that autophagy contributes to the biogenesis of Triton WR 1339-filled lysosomes. This has been discussed in a previous report in more detail[9].

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Experimentals

The lysosomal membrane fraction of livers of Triton WR 1339-treated rats was prepared as reported previously[1]. The purity of the intact lysosomes and of the membrane fraction was checked by marker enzymes[1,10] and by electron microscopy. The extraction of ubiquinone was achieved either with chloroform/methanol 2:1 from a briefly sonicated aqueous dispersion of lysosomal membranes or with n-hexane after the addition of two volumes of methanol[3]. From the water-washed lipid extracts ubiquinone was purified by repeated preparative thin-layer chromatography (silica gel H, solvent system n-hexane/diethyl ether 7:3). By this procedure about 0.1 mg of ubiquinone was isolated from 19 mg of lysosomal membrane protein. This compound was shown to be pure by thin-layer chromatography using two solvent systems (n-hexane/diethyl ether 7:3, chloroform/benzene 1:1). It was used as a reference substance for the isolation of this compound from total rat liver in amounts suitable for identification.

75 g of rat liver (fresh weight) was extracted with chloroform/methanol 2:1. The neutral lipid fraction was prepared by elution of a silicic acid column with chloroform. The eluate was evaporated under nitrogen and the dry residue was dissolved in n-hexane for further separation of the neutral lipids by column chromatography on silicic acid. Fractions were eluted with n-hexane and with n-hexane containing increasing

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concentrations of diethyl ether. Ubiquinone was eluted with 5% diethyl ether in n-hexane. Minor impurities, mainly triacylglycerols, disappeared on repeated preparative thin-layer chromatography. By this procedure 12.9 mg of an orange coloured material was obtained. Crystallization was possible from hot ethanol (m.p. 36–38°C). Identification was achieved by UV spectroscopy in ethanol and n-hexane and by infrared spectroscopy (Perkin Elmer 402 Ultraviolet-Visible Spectrophotometer and Perkin Elmer 257 Grating Infrared Spectrophotometer).

Quantitative determination of ubiquinone in n-hexane extracts of lysosomal membranes was performed by a microadaptation of the method of Crane[11]. Portions of the extracts were transferred to quartz microcuvettes, evaporated in a stream of nitrogen and dissolved in 0.5 ml of ethanol (Uvasol, Merck, Darmstadt). The ubiquinone content was calculated by measuring the decrease in optical density at 275 nm after reduction of ubiquinone with 2.5 µl of a freshly prepared solution of 0.3% $NaBH_4$ in water using a molar extinction coefficient of $\Delta E_{ox-red} = 12250\ cm^2/mol$[12,13]. Determination of the isoprenoid side chain length was possible by thin-layer chromatography on paraffin-treated silica gel plates according to the method described by Wagner and Dengler[2] using synthetic Q_{10} as a reference compound.

NADH-dehydrogenase activity was assayed according to Mackler[14] using 2,6-dichlorophenolindophenol (Fluka, Switzerland) as electron acceptor. The assay system contained in a total volume of 3.0 ml: 0.2 ml of 1M phosphate buffer pH 7.8, 0.03 ml of a 0.01% solution of 2,6-dichlorophenolindophenol (sodium salt), 0.01 ml of 1% NADH solution and 50–200 µg of protein. The reduction of 2,6-dichlorophenolindophenol was followed by the decrease in extinction at 600 nm.

"Lysosomal free mitochondria" were prepared from livers of Triton WR 1339-treated rats as described previously[1]. The final supernatant (105000 × g, 60 min) was designated the "cell sap". Protein was determined by the Lowry[15] method. For electron microscopy the lysosomal membrane fraction was embedded in 2% agar solution[16]. The agar was cut into small cubes for fixation with Palade's veronal-acetate buffered osmium tetroxide fixative[17]. Embedding was performed, after ethanol dehydration, with Epon 812. Sections were cut with a LKB Ultramicrotome. Staining was performed according to Millonig[18]. Electron micrographs were taken with a Philips EM 300 electron microscope.

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