OXIDATION-REDUCTION LEVELS OF UBIQUINONE (COENZYME Q) IN DIFFERENT METABOLIC STATES OF RAT LIVER MITOCHONDRIA

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The precise role of ubiquinone (coenzyme Q) in the phosphorylating electron transport system is not yet understood. Hatefi (1959) has measured the steady-state oxidation-reduction levels of the quinone in beef heart mitochondria and suggested that it may be intimately concerned with one of the sites of oxidative phosphorylation. In the present paper the results of a study of the oxidation-reduction levels of ubiquinone in rat liver mitochondria are presented and discussed in relation to its possible mode of action.

Rat liver mitochondria were isolated in 0.25 M sucrose essentially by the method of Schneider and Hogeboom (1950) and were used immediately after preparation. P/O and respiratory control ratios were determined polarographically (Chance and Williams, 1955a). Oxidation-reduction levels of ubiquinone were measured by methods described previously (Pumphrey and Redfearn, 1960; Redfearn and Pumphrey, 1960). Cytochrome concentrations were measured by modifications of the methods of Chance and Williams (1955b) and Green et al. (1959).

In a previous report (Pumphrey and Redfearn, 1960) it was stated that the determination of ubiquinone concentration in rat liver mitochondria was difficult owing to the presence of vitamin A. It has now been found that when liver mitochondria from hooded

rats from an inbred strain at Liverpool are analysed by the procedure described, there is little or no interference from vitamin A.

The relative concentrations of the cytochromes and ubiquinone were determined in a number of mitochondrial preparations and the mean molar ratio cytochrome $\underline{a}$: $\underline{b}$: $\underline{c}_1$: $\underline{c}$: UQ was 1 : 1 : 1.3 : 0.9 : 11. Thus ubiquinone is present in considerable excess compared with the other carriers.

Measurement of oxygen consumption of the mitochondria at room temperature (16-18°) in the presence of ADP and inorganic phosphate gave values of 3.8, 1.0, 1.4 and 0.4 µatoms O_2/hr./mg. protein for succinate, β-hydroxybutyrate, α-ketoglutarate and internal substrate respectively; in the absence of ADP the corresponding values were 0.6, 0.1, 0.2 and 0.2 µatoms O_2/hr./mg. protein. P/O ratios were 1.8, 3.0 and 3.0 for succinate, β-hydroxybutyrate and α-ketoglutarate respectively.

The oxidation-reduction levels of ubiquinone under various metabolic conditions of the mitochondria are shown in Table I. A small portion of the total ubiquinone, varying between 10 and 20% in different preparations, appeared to be enzymically inactive; in the presence of antimycin A, which inhibits ubiquinol oxidation, only 80-90% of the total ubiquinone is reducible enzymically. Thus in the absence of ADP or inorganic phosphate, succinate is actually giving complete reduction and β-hydroxybutyrate about 80% reduction of the enzymically active ubiquinone.

Omission of ADP from the reaction mixture always resulted in an increased reduction of ubiquinone; this result is qualitatively similar to that of Hatefi (1959). Also when inorganic phosphate was omitted and replaced by tris-HCl buffer there was an increased reduction, usually of the same magnitude as that obtained in the absence of ADP. This result is contrary to that of Hatefi (1959)

TABLE I

Steady-state Oxidation-Reduction Levels of Ubiquinone in Different Metabolic States of Rat Liver Mitochondria.

Preparation number	Ubiquinone concentration (μmoles/g. protein)	Substrate	Steady-state percentage reduction of total ubiquinone			
			+Pi +ADP +O_2	+Pi +O_2	+ADP +O_2	+O_2
14	1.3	endogenous	38	45	44	-
		succinate	72	80	80	82
15	1.6	endogenous	45	-	-	-
		β-hydroxy-butyrate	44	64	68	-
		succinate	80	85	87	88
17	1.3	endogenous	33	-	-	-
		β-hydroxy-butyrate	30	53	-	-
		succinate	69	81	84	-
18	2.2	endogenous	63	-	-	-
		β-hydroxy-butyrate	63	72	72	-
		succinate	86	89	88	-
19	1.8	endogenous	35	-	-	-
		α-ketoglutarate	48	67	69	85

Reaction mixture as follows : sucrose, 107 mM; KCl, 25 mM; Na_2HPO_4-KH_2PO_4, pH 7.4, 12.5 mM (or tris-HCl, pH 7.4, 18.8 mM); ADP, 18 mM; sodium β-hydroxybutyrate, 4.5 mM; sodium α-ketoglutarate, 4.5 mM; sodium succinate, 3.0 mM; mitochondrial protein, approx. 6 mg./ml. Total volume, 1.4 ml. Mixture aerated for 30 sec. Temp. 17-20°.

who found that when phosphate was replaced by tris there was no reduction of ubiquinone. It is possible that Hatefi's results can be explained on the basis of a non-enzymic oxidation of ubiquinol during the isolation procedure; the susceptibility of ubiquinol to oxidation may be increased when phosphate is absent, particularly when heat treatment is used to stop the reaction.

The changes in the steady-state oxidation-reduction levels of

ubiquinone in the different metabolic states of mitochondria are in line with those of the other respiratory chain components and with the mechanism of oxidative phosphorylation suggested by Chance and Williams (1956). It is possible that ubiquinone may function in oxidative phosphorylation in a high-energy form, $UQH_2 \sim I$, as suggested by Chance (1960) and Hatefi (1959) but no evidence for the existence of such a compound has yet been obtained.

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