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Saturation kinetics of coenzyme Q in NADH and succinate oxidation in beef heart mitochondria

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The saturation kinetics of NADH and succinate oxidation for Coenzyme Q (CoQ) has been re-investigated in pentane-extracted lyophilized beef heart mitochondria reconstituted with exogenous CoQ_{10}. The apparent 'K_m' for CoQ_{10} was one order of magnitude lower in succinate cytochrome *c* reductase than in NADH cytochrome *c* reductase. The K_m value in NADH oxidation approaches the natural CoQ content of beef heart mitochondria, whereas that in succinate oxidation is close to the content of respiratory chain enzymes.

Coenzyme Q; NADH cytochrome *c* reductase; Kinetics; Beef heart mitochondrion

1. INTRODUCTION

Evidence is accumulating that a deficiency of Coenzyme Q (ubiquinone, CoQ) accompanies several pathological alterations and that administration of exogenous CoQ is beneficial for such conditions [1]. CoQ is well recognized as a mobile, substrate-like, redox component of the mitochondrial respiratory chain, situated between flavoprotein dehydrogenases and the cytochrome bc_1 complex (or Complex III) [2].

The function of CoQ as a mobile substrate-like component in the respiratory chain has long been established; Kröger and Klingenberg [3] have demonstrated that CoQ behaves as a kinetically homogeneous pool, and this behavior has been confirmed in several laboratories [4–6]. Norling et al. [6] in a study of pentane-extracted and reconstituted mitochondria established that the CoQ concentrations yielding half-maximal velocity of electron transfer in both NADH and succinate oxidation by molecular oxygen were in the range of 2 nmol per mg protein in bovine heart submitochondrial particles. These concentrations are not far from the contents reported for CoQ in bovine heart mitochondria and raise the question whether CoQ concentration in the membrane is kinetically saturating.

In the light of these considerations and of the renewed interest in CoQ membrane concentration for its biomedical implications [1], we have decided to re-investigate the saturation kinetics of CoQ in the NADH and succinate to cytochrome *c* span of the respiratory chain, in order to involve only each couple of enzyme complexes connected by the CoQ pool (Complex I or II and Complex III), without the complicating presence of additional possible rate-limiting steps as cytochrome *c* and cytochrome oxidase when molecular oxygen is used as the electron acceptor, as in [6].

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2. MATERIALS AND METHODS

Ubiquinone homologs were kindly provided by Eisai Co. (Tokyo, Japan) and stored in absolute ethanol at −20°C.

Beef heart mitochondria were prepared from slaughterhouse material by a small-scale procedure [7]. In order to extract CoQ, the membranes were lyophilized and treated with pentane for extraction and reconstitution essentially as described by Norling et al. [6].

NADH cytochrome *c* reductase and succinate cytochrome *c* reductase were assayed by following the reduction of cytochrome *c* in a Jasco 7850 UV-VIS spectrophotometer, as described elsewhere [8]. NADH- and succinate-linked oxygen consumptions were followed in a Clark oxygen electrode [9].

Ubiquinone content in mitochondrial membranes was determined by reverse-phase HPLC analysis [10] in a Waters apparatus after methanol–light petroleum extraction as described elsewhere [11]. Protein was determined by the method of Lowry et al. [12].

Cytochrome *b* was determined by the dithionite-reduced minus oxidized spectrum according to [8].

3. RESULTS

The CoQ content of the mitochondria used throughout this study (obtained from slaughterhouse material in the course of several months) was variable and oscillated in the range of 1–4 nmol/mg protein; this variability was maintained if the concentration was expressed relative to other mitochondrial respiratory chain components, e.g. cytochrome *b*. The same contents were found in the mitochondria after they had undergone lyophilization (Table I).

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Table I

Ubiquinone and cytochrome content of lyophilized beef heart mitochondria from different preparations

	nmol · mg^{-1}
Ubiquinone	2.4 ± 1.0 (5)
Cytochrome *b*	0.57 ± 0.03 (3)

Values are means ± S.D. for the number of experiments expressed between parentheses.

NADH and succinate cytochrome *c* reductase activities were assayed in a number of membrane samples that had undergone different extents of reconstitution with CoQ_{10}; in each case the activity was plotted as a function of the quinone that had been bound and determined by HPLC analysis after methanol–light petroleum extraction of each sample. The results of a number of typical experiments are shown in Fig. 1. From its analysis we can observe the following: (i) the apparent K_m for CoQ (strictly kinetically expressed as the concentration giving half-maximal velocity) is one order of magnitude lower in succinate cytochrome *c* reductase than in NADH cytochrome *c* reductase; (ii) the 'K_m' values are close to the physiological CoQ content in the membrane in the case of NADH oxidation, but are close to the enzyme content in the membrane (as indicated by cytochrome *b* content, see Table I) in the case of succinate oxidation.

These results are strikingly different from those of Norling et al. [6] for succinate oxidation; since those authors assayed oxidative activities as oxygen consumption, we have also determined the CoQ saturation kinetics in the NADH to oxygen and in the succinate to oxygen spans; the results (Table II) are substantially similar to those found for the cytochrome *c* reductase assays.

In order to better define the shape of the reconstitution curve for CoQ in succinate cytochrome *c* reductase, we have titrated this activity in CoQ-depleted particles with CoQ_3, a CoQ homolog having full reconstitutive activity for this system [13] and sufficiently water soluble to be added directly to the assay medium. Fig. 2 shows the V_{max} is reached hyperbolically, suggesting that Complexes II and III are connected by a homogeneous CoQ pool; a similar titration of NADH cytochrome *c* reductase with CoQ_3 revealed a lack of full reconstitution (cf. [13]) due to the well known inhibition of Complex I activity by short chain CoQ homologs [14].

Table II

Kinetic constants for NADH and succinate oxidase in pentane-extracted and CoQ-reconstituted beef heart mitochondria

	V_{max} μg atoms $O_2 \cdot min^{-1} \cdot mg^{-1}$)	K_m for CoQ nmol·mg^{-1}
NADH oxidase	0.52	1.7
Succinate oxidase	0.12	0.28

4. DISCUSSION

The composite activities of NADH- and succinate cytochrome *c* reductase through Coenzyme Q are functions of the rates of reduction and oxidation of the quinone according to the CoQ pool equation [3], but also of CoQ concentration in the membrane [15,16]; a 'K_m' value for total CoQ in each of these systems represents a poised value which depends upon the relative V_{max} of the enzymes involved and the dissociation constants of quinone/quinol from the complexes [15]. Oper-

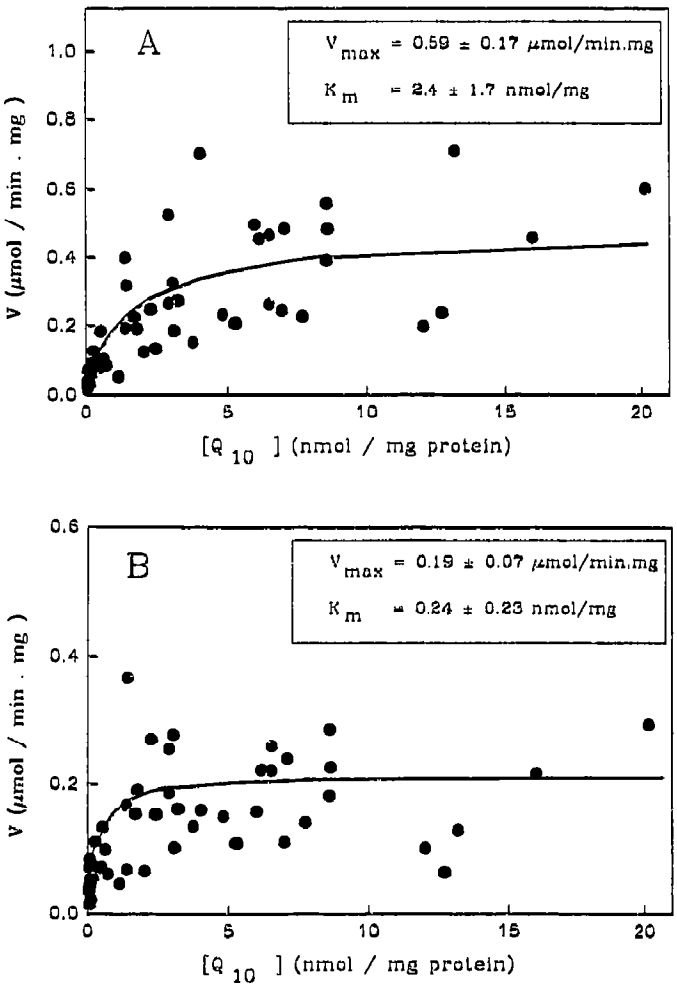

Fig. 1. Saturation curves of NADH- and succinate cytochrome *c* reductase in pentane-extracted mitochondria with CoQ_{10}. The extracted mitochondria were reconstituted as described in the text. Each curve is the combination of 6 different extraction and reconstitution experiments. (A) NADH cytochrome *c* reductase; (B) succinate cytochrome *c* reductase. The V_{max} and K_m values in the insets are means ± S.D. of the V_{max} and K_m obtained in each separate reconstitution experiment.

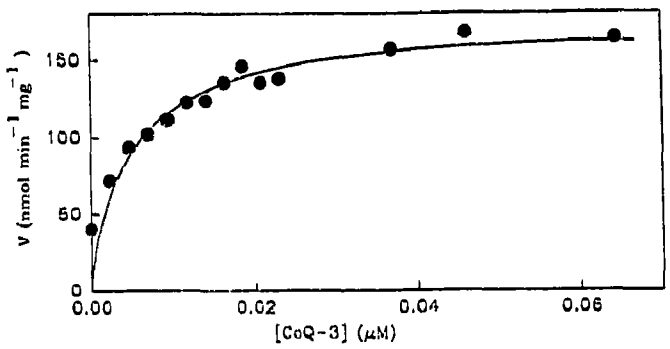

Fig. 2. Saturation curve of succinate cytochrome *c* reductase of pentane-extracted mitochondria with CoQ_3. The residual level of CoQ_{10} in the extracted mitochondria was 0.045 nmol/mg protein; the V_{max} (0.17 μmol·min^{-1}·mg^{-1}) was comparable with the activity of the parent lyophilized mitochondria (0.13 μmol·min^{-1}·mg^{-1}).

ationally, however, the 'K_m' values obtained are those CoQ concentrations yielding a half-maximum rate of cytochrome *c* reduction; these are of physiological importance in that they define the extent of saturation with CoQ of this segment of the respiratory chain.

In pentane-extracted and CoQ-reconstituted lyophilized mitochondria we have found that the average K_m value (as defined above) for CoQ_{10} in NADH cytochrome *c* reductase is close to 2.5 nmol/mg protein; this value may be calculated to correspond to a concentration of 5 mM CoQ in the membrane phospholipids, assuming a uniform distribution of CoQ in the whole phospholipid bilayer and that 1 mg phospholipid occupies 1 μl volume [17].

This K_m value is quite close to the highest CoQ concentrations found in our mitochondrial preparations. This means that most preparations of mitochondria are deficient in CoQ in terms of kinetic saturation of NADH oxidation. We do not have data for human heart mitochondria, but the lowered CoQ values found in cardiac patients [1] may be reasoned to result in a lowered rate of NADH oxidation.

A different case has been found for succinate cytochrome *c* reductase, where the K_m value for CoQ_{10} is one order of magnitude lower, and close to the concentration of respiratory enzymes in the membrane. Although a simple explanation is an exceedingly low K_m of Complex II for CoQ, the latter observation may suggest that, in this system, CoQ is not part of a homogeneous pool but is subcompartmentalized in a local pool or in a stoichiometric association with Complexes II and III. The shape of the CoQ saturation curve, obtained using CoQ_3 for technical reasons, supports the former possibility. Direct association of Complexes II and III (contrary to Complexes I and III) has been repeatedly advanced (cf. [18]). Gwak et al. [19] reported that Complexes II and III undergo preferential associations in phospholipid vesicles, and the resulting succinate cytochrome *c* reductase supercomplex containing 1 mol CoQ per mol, is fully active, in agreement with our results. The high affinity for CoQ of the succinate system explains why in mitochondria from neuroblastoma cells, a lowered CoQ biosynthesis had no effect on succinate oxidation [20].

The reason for the discrepancy of the K_m values for CoQ in succinate oxidation between this work and that of Norling et al. [6] is not clear, particularly considering we have used the same experimental conditions; it is not due to the different electron acceptor used (cytochrome *c* or oxygen) because in our preparations the values are identical with both acceptors. If the low K_m values are the expression of stoichiometric association of Complexes II and III and CoQ, then conditions must exist where this association is broken and normal pool behavior is resumed.

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