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# Interaction Between Ubiquinone and ATPase in Mitochondrial Membranes[1]

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## Abstract

The extraction of ubiquinone from mitochondrial membranes produces alterations of ATPase activity including a reversible loss of oligomycin sensitivity which is restored by long-chain Q-homologs. Short-chain ubiquinones like $Q_3$ produce a loss of oligomycin and dicyclohexylcarbodiimide (DCCD) sensitivity in submitochondrial particles. The effect shows uncompetitive or noncompetitive kinetics with respect to oligomycin or DCCD respectively. Long-chain ubiquinones have a competitive effect with $Q_3$, thus restoring oligomycin sensitivity; they behave, however, in about the same way as $Q_3$ in lowering the DCCD sensitivity in submitochondrial particles. On the basis of these observations we suggest that ubiquinone may be a physiological modulator of ATPase activity in the mitochondrial membrane.

**Key Words:** Ubiquinone; ATPase; mitochondrial membrane.

## Introduction

The ubiquinone pool of the mitochondrial membrane plays a central role in oxidative phosphorylation, but its exact functional aspects are still unclear.

The fact that endogenous $Q_{10}$ is about 10-fold more concentrated than other components of the respiratory chain (Crane, 1977) suggests that it may undergo a large complex of interactions in the lipid–protein environment of the membrane.

It is therefore likely that connections may also be present between ubiquinone and the bulky Complex V (ATPase) which is about 16% of the

[1]Abbreviations used: BHM, beef heart mitochondria; DCCD, dicyclohexylcarbodiimide; ETP, electron transfer particles (submitochondrial particles); Q, ubiquinone.

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total protein content in the inner mitochondrial membrane (Ozawa and Asai, 1973).

It is known that Complex V is constituted by a soluble portion (called $F_1$) protruding into the mitochondrial matrix and containing the catalytic site of the enzyme, and by a hydrophobic portion ($F_0$) inserted in the membrane, which confers oligomycin and DCCD sensitivity to ATPase (Pedersen, 1974).

Preliminary studies from our laboratories (Bertoli *et al.*, 1978) have pointed out that ubiquinone is required for the inhibition of mitochondrial ATPase by energy-transfer inhibitors.

Lopez-Moratalla *et al.* (1978) have found that $Q_{10}$ stimulates the activity of isolated soluble oligomycin-insensitive ATPase ($F_1$) and suggested that the quinone may be an allosteric modulator of ATPase activity.

For a better understanding of the problem, we have carried out a kinetic study on the effects of endogenous and exogenous ubiquinones on ATPase activity in mitochondrial membranes.

In the study we have mainly investigated the influence of ubiquinones on the sensitivity of ATPase to energy-transfer inhibitors like oligomycin and DCCD. The results suggest that there may be interactions between coenzyme Q and the ATPase complex although it cannot be determined at present whether they are direct or indirect. These observations are also related to previous studies on the uncoupling effect of short-chain ubiquinones (Bertoli *et al.*, 1978; Jacobs and Crane, 1960).

## Materials and Methods

Beef heart mitochondria (BHM) were prepared by a large-scale procedure (Smith, 1967) and submitochondrial particles (ETP) were obtained by sonication of BHM according to Hansen and Smith (1964).

Q-depleted mitochondria were obtained by pentane extraction after lyophilization in accordance to Szarkowska (1966) with some modifications as described by Bertoli *et al.* (1978). ETP or BHM were suspended in 10 mM $MgCl_2$ prior to lyophilization, and pentane extraction was performed by shaking in the cold three times for a total of 3 h.

Protein was determined by the biuret method (Cleland and Slater, 1953).

ATPase activity was assayed in a medium containing 0.1–0.3 mg of mitochondrial protein, 6 mM ATP, 3 mM $MgCl_2$ and 50 mM Tris-acetate, pH 8.5, in a final volume of 1 ml (Bertoli *et al.*, 1978). The phosphate liberated was detected spectrophotometrically. (Griffiths and Hought, 1974). Q-homologs dissolved in ethanol were added to a final concentration of

0.2–0.4 mM and were preincubated 10 min either before or after the addition of inhibitors. Care was taken that all samples in each experiment contained the same amounts of ethanol (usually 2–4% by volume). Such amounts were shown in separate experiments not to affect ATPase activity and its sensitivity to inhibitors.

The ATPase activity of BHM and ETP was sensitive to oligomycin and DCCD up to 80–90%. In most experiments, however, inhibitors were added in such amounts as to induce a lower degree of inhibition, since under these conditions the experiments usually showed more appreciable differences (Bertoli *et al.*, 1978).

For investigating the amounts of exogenous quinones incorporated into mitochondrial membranes under the above conditions we used a double extraction method (Degli Esposti *et al.*, 1979). After a pentane washing of the Q that was not incorporated in mitochondria, the concentration of ubiquinone present in mitochondria was determined by means of the procedure of Kröger and Klingenberg (1978). Controls showed that pentane did not wash away endogenous ubiquinone.

## Results

### *Effect of Q Extraction on ATPase activity*

Pentane extraction of ubiquinone produces some modifications of mitochondrial ATPase activity, both in submitochondrial particles and in intact mitochondria. In most experiments we have observed that the enzymatic activity was clearly lower in Q-depleted BHM or ETP than in the corresponding lyophilized BHM or ETP (Table I). The addition of exogenous $Q_{10}$ or of mixed phospholipids (asolectin purified by ether extraction and centrifugation) does not, however, completely restore the ATPase activity in pentane-extracted BHM (Table II), showing that a certain extent of irreversible denaturation has occurred. Kinetic studies in lyophilized mitochondria have

**Table I.** Effect of Q Extraction on ATPase Activity of Lyophilized BHM

| | ATPase activity ($\mu$mol $P_i$/min · mg protein) | |
|---|---|---|
| Treatment | ETP | BHM |
| None | 1.36 | 1.28 |
| Lyophilized | 1.68 | 1.92 |
| Lyophilized and pentane extracted | 0.92 | 1.36 |

**Table II.** Effect of Pentane Extraction on Oligomycin[a] Sensitivity in Lyophilized BHM

| Experiment | BHM | Addition | ATPase activity ($\mu$mol $P_i$/min · mg protein) − Oligo | + Oligo | % Inhibition |
|---|---|---|---|---|---|
| 1. | Unextracted | — | 1.2 | 0.64 | 46.7 |
| | Q-Depleted | None | 1.2 | 1.08 | 10 |
| | Q-Depleted | 106 nmol $Q_3$ | 0.84 | 0.64 | 23.8 |
| | Q-Depleted | 87 nmol $Q_7$ | 1.32 | 0.8 | 39.4 |
| 2. | Q-Depleted | None | 0.62 | 0.44 | 29 |
| | Q-Depleted | 310 nmol $Q_{10}$ | 0.60 | 0.35 | 42 |
| | Q-Depleted | 308 nmol asolectin | 0.72 | 0.61 | 15 |

[a]Oligomycin is 0.02 $\mu$g/ml in Experiment 1 and 0.04 $\mu$g/ml in Experiment 2.

shown that ubiquinone extraction induces a decrease of $V_{max}$ and reduces to half the $K_m$ for ATP (Fig. 1). Such uncompetitive kinetics appear very similar to those obtained by Parenti-Castelli *et al.* (1979) by perturbing the membrane environment with phospholipase $A_2$ and other agents. It is possible that pentane extraction partially damages the phospholipid membrane integrity.

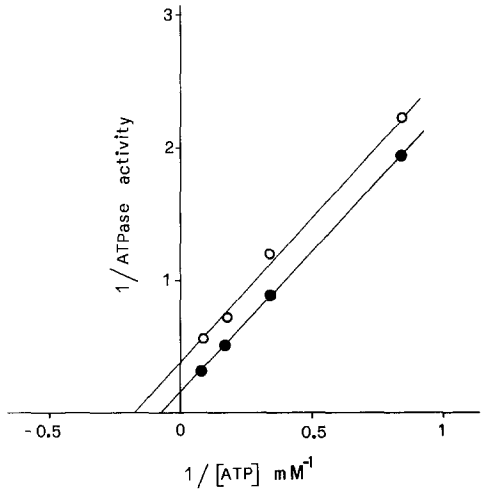


**Fig. 1.** Double reciprocal plots of ATPase activity in lyophilized BHM and in Q-depleted BHM. ●, lyophilized BHM; ○, lyophilized and pentane-extracted BHM.

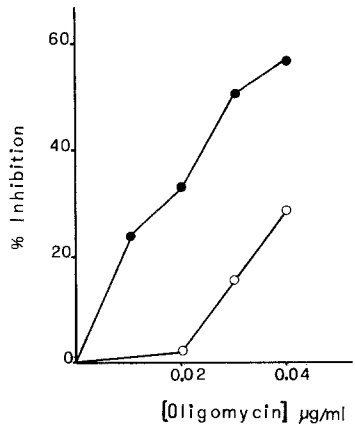


**Fig. 2.** Effect of Q extraction on the oligomycin sensitivity of lyophilized BHM. ●, unextracted BHM; ○, pentane-extracted BHM.

## *Effect of Q Depletion on the Sensitivity of ATPase to Oligomycin and DCCD*

In previous studies it was observed that in Q-depleted ETP and in Q-depleted BHM (Bertoli *et al.*, 1978) there is a reversible loss of oligomycin sensitivity of ATPase. (Fig. 2). This effect does not depend on a modification of the inhibition mechanism caused by the extraction of some lipid components of the membrane, because addition of long-chain ubiquinones restores

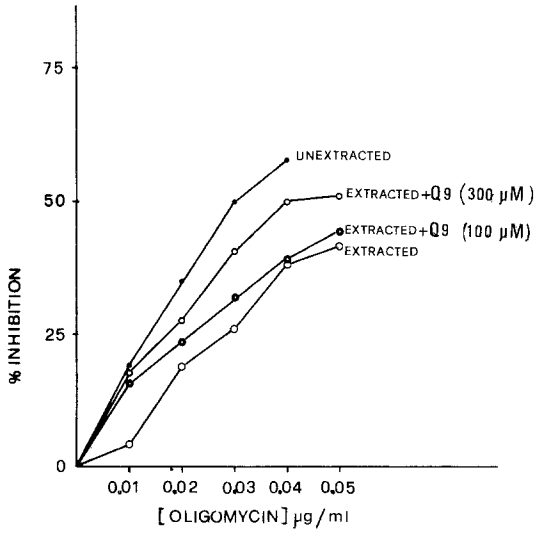


**Fig. 3.** Effect of Q extraction on the oligomycin sensitivity of lyophilized ETP.

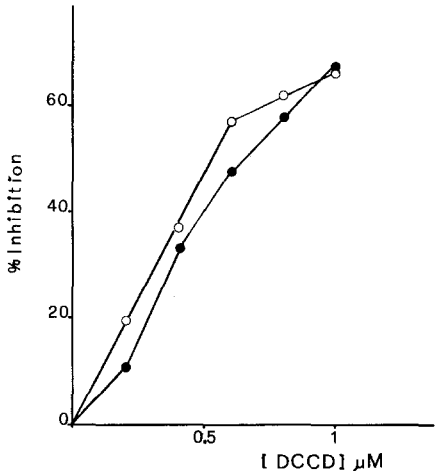


**Fig. 4.** Effect of Q extraction on DCCD sensitivity of lyophilized BHM. ●, unextracted BHM; ○, pentane-extracted BHM.

normal oligomycin sensitivity (Fig. 3, for ETP) whereas addition of mixed phospholipids, on the contrary, slightly reduces the extent of inhibition (Table II). Short-chain ubiquinones like $Q_3$, at the same concentrations, do not restore oligomycin sensitivity of ATPase (Table II).

Differently from oligomycin, the DCCD sensitivity of ATPase is not significantly affected by Q depletion, as shown in Fig. 4.

### *Effect of $Q_3$ on Oligomycin Sensitivity of ATPase*

Prior to investigating the effect of exogenous ubiquinones on ATPase activity, we have determined the amounts of the quinones incorporated in the mitochondrial membranes under the same assay conditions.

**Table III.** Extent of Incorporation of Some Ubiquinones in ETP[a]

| Ubiquinone | nmol/mg protein | % Incorporation |
|---|---|---|
| $UQ_1$ | none | ~0 |
| $UQ_3$ | 18 | 6 |
| $UQ_4$ | 36 | 12 |
| $UQ_5$ | 55 | 18 |
| $UQ_8$ | 53 | 18 |
| $UQ_9$ | 54 | 18 |
| $UQ_{10}$ | 67 | 22 |

[a] Ubiquinones are added to a final concentration of 0.3 mM. The incorporation was investigated as described in Methods. Each sample contained 2 mg of protein in a total volume of 1 ml.

The results, reported in Table III, show that $Q_3$ is incorporated only to an extent of 6%, corresponding to an intramembrane concentration of 18–20 nmoles/mg protein. Slightly higher incorporations are found for longer-chain quinones.

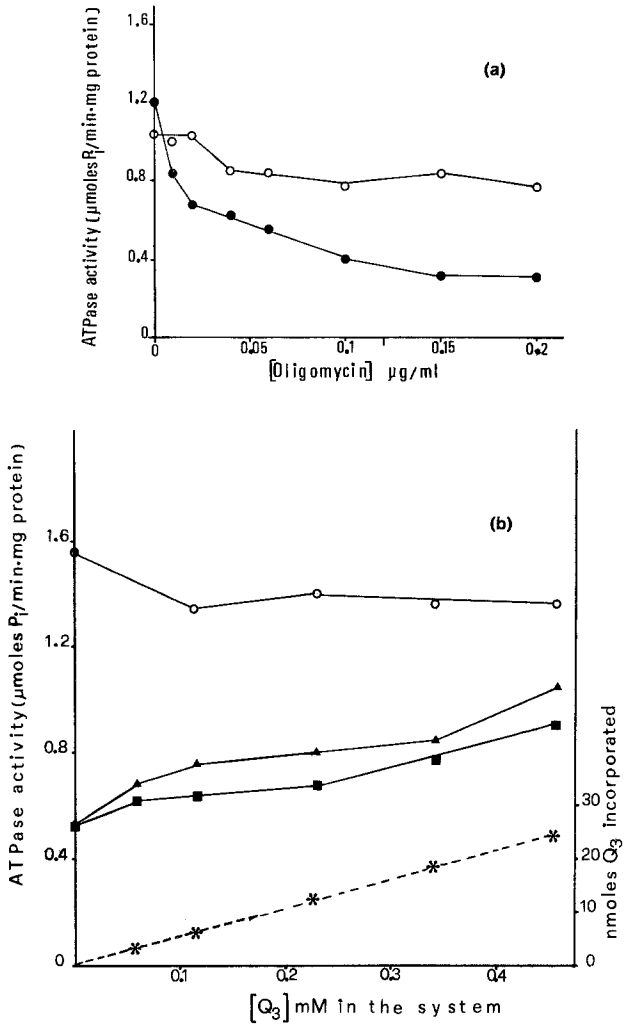


**Fig. 5.** Effect of $Q_3$ on oligomycin sensitivity of ATPase in ETP. (a) As a function of oligomycin concentration. ●, control; ○, in the presence of 0.34 mM $Q_3$. (b) As a function of $Q_3$ concentration. ○, control without oligomycin; ■, oligomycin (0.08 μg/ml) added before $Q_3$; ▲, oligomycin (0.08 μg/ml) added after $Q_3$; *, amounts of $Q_3$ incorporated per milligram of membrane protein, calculated as described in the methods.

Among the exogenous quinones tested, only $Q_3$ induces a clear loss of oligomycin sensitivity of ATPase in ETP (Bertoli *et al.*, 1978). In fact, when this quinone is incorporated at about three to four times the concentration of endogenous $Q_{10}$ (cf. Table III), the inhibition by oligomycin undergoes a large decrease (Fig. 5a), even if $Q_3$ is added after the inhibitor (Fig. 5b).

This effect of $Q_3$ can be reversed by the addition of $Q_7$, but only if the ubiquinones are added before oligomycin (Table IV).

We have carried out a study of the inhibition kinetics of oligomycin in the presence of $Q_3$, and it was found that $Q_3$ decreases both $K_I$ and maximal percentage inhibition, behaving like an uncompetitive factor toward oligomycin sensitivity of ATPase (Fig. 6). On the contrary, $Q_7$ does not modify the oligomycin inhibition kinetics.

### *Effect of $Q_3$ on DCCD Sensitivity of ATPase*

$Q_3$ also abolishes DCCD sensitivity of ATPase in ETP (Fig. 7), and this effect is observed even in DCCD-pretreated particles.

$Q_3$ addition affects the inhibition kinetics of DCCD in a noncompetitive way, as it does not change the $K_I$ while clearly decreasing the maximal percentage of inhibition. Such an effect is still evident even if DCCD is already bound to $F_0$ in submitochondrial particles pretreated with the inhibitor for 10 min, as shown in Fig. 8.

Long-chain ubiquinones like $Q_7$ are not able to restore the normal DCCD inhibition altered by $Q_3$ (Table V). In fact, all Q-homologs tested and also $\alpha$-tocopherol lower the DCCD sensitivity of ATPase in ETP.

Among the isoprenologs tested, only $Q_3$, however, is able to partially remove the inhibition induced by DCCD already bound to submitochondrial particles, whereas all other Q-homologs do not have any effect under such condition. The specific action of $Q_3$ can be interpreted as a disorganization of the DCCD binding site in $F_0$, whereas the effects of the other tested lipophilic molecules with isoprenoid side chains may be ascribed to a blockage of

**Table IV.** Effect of $Q_3$ and $Q_7$ on the Sensitivity to Oligomycin[a] of ATPase in ETP

| Addition (nmol) | % Inhibition of ATPase activity | |
|---|---|---|
| | Ubiquinones added before oligomycin | Ubiquinones added after oligomycin |
| None | 42.3 | 47 |
| + $Q_3$ (342) | 8.6 | 25.6 |
| + $Q_7$ (352) | 38.4 | 36 |
| + $Q_3$ + $Q_7$ (respectively 342 and 352) | 30.6 | 26.7 |

[a]Oligomycin is 50 ng/ml.

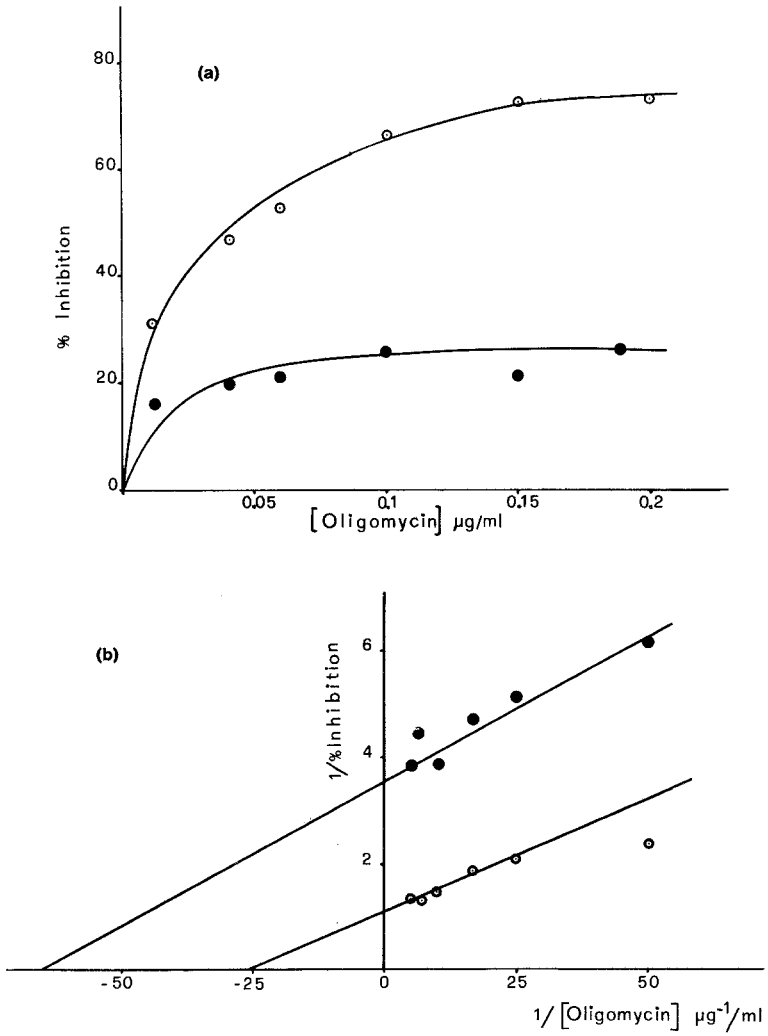


**Fig. 6.** Direct plots (a) and double reciprocal plots (b) of oligomycin sensitivity of ATPase in the presence of $Q_3$ in ETP. ⊙, control; ● +344 nmol of $Q_3$.

DCCD binding to $F_0$. All the above effects are not due to direct chemical interactions with the carbodiimide in the medium, as demonstrated by control experiments where DCCD was preincubated with ubiquinones.

### *Specificity of the effect of $Q_3$*

In order to determine if the above effect of $Q_3$ is really specific, we have tested other lipophilic substances like ergocalcipherol, cholesterol, and $\alpha$-

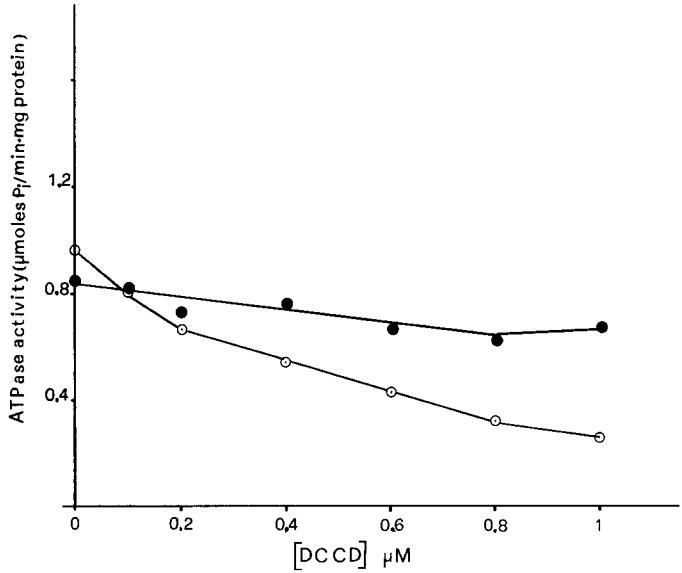


**Fig. 7.** Effect of DCCD on ATPase activity of ETP in the presence of $Q_3$ added before the inhibitor. ⊙, control; ● +344 nmol of $Q_3$.

tocopherol under the same assay conditions. As shown in Table VI, both cholesterol and ergocalcipherol lower the oligomycin sensitivity of ATPase as $Q_3$ does, but they also produce a relevant loss of the enzymatic activity so that their effect cannot be fully compared with the action of $Q_3$ or other quinones. Also detergents like Triton X-100 and sodium dodecyl sulfate (SDS) produce alterations of ATPase activity which are not comparable with the effect of ubiquinone-3.

## Discussion

The extraction of endogenous $Q_{10}$ and the addition of exogenous $Q_3$ induce a decrease of oligomycin sensitivity of mitochondrial ATPase, which can be restored by long-chain quinones like $Q_7$. Moreover also the ATP–$P_i$ exchange reaction is decreased by the extraction of ubiquinone, while long-chain Q-homologs can restore normal rates of ATP–$P_i$ exchange (E. Bertoli and M. Carver, unpublished). In this work we have found that Q depletion and $Q_3$ addition have also different effects, particularly on the DCCD sensitivity of ATPase. The available data allow us to postulate that exogenous ubiquinones block the accessibility of DCCD to its binding site; only $Q_3$ is able, however, to partially abolish the inhibition of DCCD already

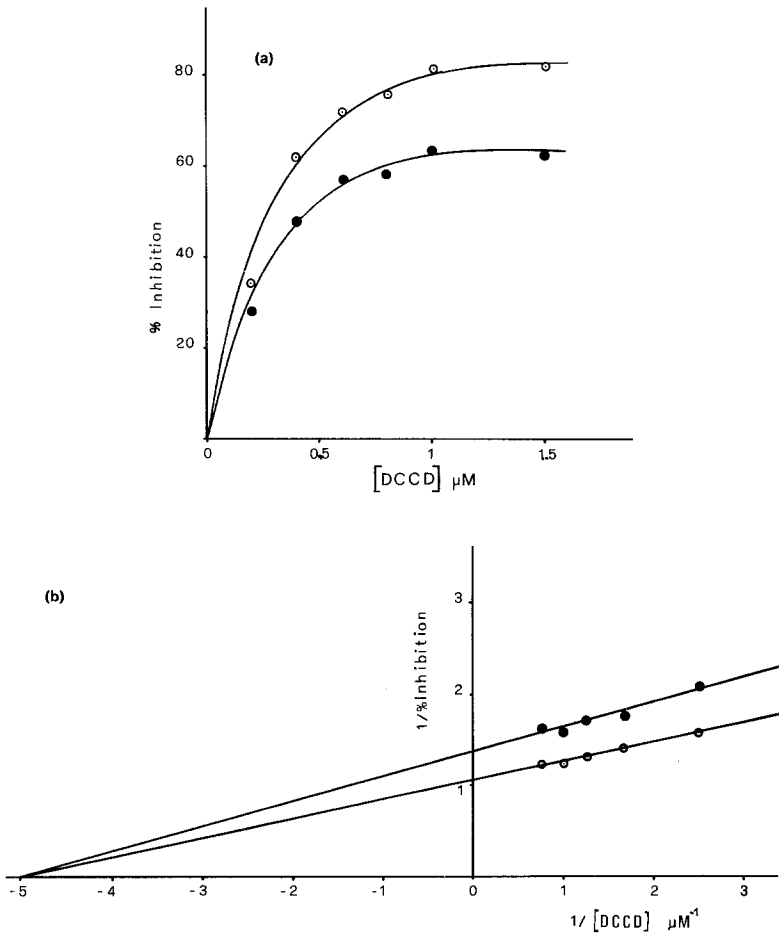


**Fig. 8.** Direct plots (a) and double reciprocal plots (b) of DCCD sensitivity of ATPase in ETP with $Q_3$ added after the inhibitor. ⊙, control; ● +344 nmoles of $Q_3$.

bound to submitochondrial particles (cf. Fig. 8). The hypothesis advanced by Bertoli *et al.* (1978) of a direct involvement of ubiquinone in the energy-transfer pathway from the respiratory chain to ATPase does not therefore offer a satisfactory explanation of all the results.

A simple nonspecific detergent effect of $Q_3$ can be excluded by the following experimental evidences: (a) the effect on oligomycin sensitivity is reversed by long-chain ubiquinones; (b) other Q-homologs are not able to remove the inhibition in DCCD-pretreated particles as $Q_3$ does; (c) contrary to detergents and long-chain ubiquinones, $Q_3$ immobilizes the bilayer. (Spisni *et al.*, 1978)

**Table V.** Effect of $Q_3$ and $Q_7$ on DCCD[a] Sensitivity of ATPase in ETP

| Addition (nmol) | ATPase activity ($\mu$mol $P_i$/min · mg protein) | | |
|---|---|---|---|
| | − DCCD | + DCCD | % Inhibition |
| None | 1.08 | 0.52 | 51.8 |
| + $Q_3$ (342) | 1.0 | 0.88 | 12 |
| + $Q_7$ (352) | 1.08 | 0.8 | 25.9 |
| + $Q_3$ + $Q_7$ (respectively 342 and 352) | 1.08 | 0.88 | 18.5 |

[a]DCCD is 0.6 $\mu$M and is added after ubiquinones.

Further demonstration of the peculiarity of the $Q_3$ effects arises from our experiments with lipophilic substances similar to quinones, like tocopherol, vitamin $D_2$, and cholesterol. Such agents, under the same assay conditions, do not perturb significantly the bilayer (unpublished results from our laboratory), but affect the ATPase activity and its sensitivity to inhibitors. In particular, ergocalcipherol markedly reduces the oligomycin sensitivity of ATPase in ETP (cf. Table VI), but its mode of action should be different from $Q_3$, as it also produces a striking change of the enzymatic kinetics, behaving like an uncompetitive inhibitor toward ATP hydrolysis (C. Casali, M. Degli Esposti, E. Bertoli, G. Parenti-Castelli, and G. Lenaz, unpublished), whereas $Q_3$, at the same concentrations, does not induce significant modifications of the enzymatic kinetics (cf. Fig. 5b).

We think therefore that a possible interpretation of the results consists of a perturbation of the membrane environment caused by Q depletion or exogenous Q addition which alters some lipid–protein connections involved in ATPase activity. Such a perturbation could occur through two different mechanisms also cooperating with each other: (1) modification of the phospholipid arrangement of the membrane around Complex V; (2) interac-

**Table VI.** Effect of Some Lipophilic Substances[a] on the Oligomycin[b] Sensitivity of ATPase in ETP

| Compound (0.3 mM) | % ATPase activity | % Inhibition by oligomycin |
|---|---|---|
| None | 100 | 68 |
| $Q_3$ | 88 | 29 |
| $Q_8$ | 88 | 58 |
| Ergocalcipherol | 55 | 4 |
| Cholesterol | 24 | 30 |
| $\alpha$-Tocopherol | 77 | 55 |

[a]Each compound is dissolved in absolute ethanol at a concentration of 10 mM.
[b]Oligomycin is 0.1 $\mu$g/ml.

tion of ubiquinone with some intrinsic subunits of ATPase with consequent influence on the enzymatic activity.

The former mechanism would be in accordance with much experimental evidence about the role of phospholipids on ATPase activity (Parenti-Castelli *et al.*, 1979; Bertina *et al.*, 1975; Bruni *et al.*, 1971, 1975). The extraction of endogenous ubiquinone could change the lipid environment of Complex V, while the addition of exogenous $Q_3$ would perturb the physicochemical state of the membrane (Spisni *et al.*, 1978).

The kinetic studies reported have shown that $Q_3$ is an uncompetitive factor toward oligomycin sensitivity of ATPase; similar effects were found by lipid depletion or solvent perturbation of the membrane lipids (Parenti-Castelli *et al.*, 1979), suggesting that also the action of $Q_3$ may consist in a perturbation of the lipids.

The latter mechanism implies a direct modulation operated by ubiquinone on the ATPase activity, as an allosteric factor (Bertoli *et al.*, 1978; Lopez-Moratalla *et al.*, 1978). Little experimental evidence at this time can prove such a mechanism, which, however, is not in contrast with the available data. In fact, all the results reported in this paper could be explained by assuming that the removal or displacement by exogenous Q of the endogenous ubiquinone produces an alteration of the enzymatic activity and properties.

It is not possible at the present stage to discriminate among the two possibilities, although the effects of other lipophilic substances appear against a simple nonspecific effect on the physical state of the lipids. Such a conclusion could be strengthened also by the reversibility of the effects of $Q_3$ by addition of long-chain quinones and suggests that ubiquinone is a membrane component capable of inducing directly or indirectly a particular conformation of the mitochondrial ATPase complex.

Studies on isolated oligomycin-sensitive ATPase are under way in order to understand the involvement of ubiquinone in the ATPase activity.

## Acknowledgments

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