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Effect of Cetyltrimethylammonium on ATP Hydrolysis and Proton Translocation in the F_0–F_1 H^+-ATP Synthase of Mitochondria

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Abstract

The amphiphylic alkyl cation cetyltrimethylammonium inhibits the catalytic activity of soluble and membrane-bound F_1 in a noncompetitive fashion. In sonic submitochondrial particles the Dixon plot showed a peculiar pattern with upward deviation at cetyltrimethylammonium concentration higher than 80 μM. In membrane-bound F_1 the inhibition by cetyltrimethylammonium was potentiated by the F_0 inhibitor ologomycin. Cetyltrimethylammonium also inhibited the oligomycin-sensitive proton conductivity in F_1-containing particles but was without any effect in F_1-depleted particles. Also this inhibitory effect was potentiated by oligomycin. These results indicate functional cooperative interactions between F_0 and F_1.

Key Words: H^+-ATP synthase; mitochondrial F_0–F_1 complex; ATPase; H^+-translocation; alkyl cations.

Introduction

The H^+-ATPase complex of mitochondria consists of two structurally and functionally distinct oligomeric proteins, F_1 and F_0,[3] which are responsible for chemical catalysis and the proton-conducting activity of the complex, respectively (Papa *et al.*, 1984; Godinot and Di Pietro, 1986). The F_1 moiety

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[3] Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; F_0, membrane integral sector of mitochondrial H^+-ATPase; F_1, catalytic part of mitochondrial H^+-ATPase; F_6 and OSCP, coupling factors; ESMP, submitochondrial particles prepared in presence of EDTA; Sephadex particles, ESMP passed through a Sephadex column and deprived of F_1 inhibitor protein; USMP, Sephadex particles treated with urea.

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of the complex can easily be dissociated from the membrane as a soluble oligomer of five nonidentical protein subunits (Godinot and Di Pietro, 1986; Beechey *et al.*, 1975; Vignais and Satre, 1984) with a stoichiometry of $3\alpha 3\beta\gamma\partial\varepsilon$ (Vignais and Satre, 1984; Yoshida *et al.*, 1982). The subunit stoichiometry of the F_0-sector is more controversial (Papa *et al.*, 1984; Godinot and Di Pietro, 1986).

The two sectors are structurally connected by "the stalk" apparently formed by membrane polypeptides (OSCP, F_6) (Amzel and Pedersen, 1983; Norling *et al.*, 1984). The functional interaction between the two sectors can be analyzed by resolution and reconstitution studies (Pansini *et al.*, 1978, 1979; Norling *et al.*, 1984) and by kinetic analysis of the effect of F_1 ligands and F_0 inhibitors on ATPase activity and proton translocation (Pansini *et al.*, 1978, 1979).

In this paper we report on the inhibitory effect of cetyltrimethylammonium, an amphiphylic alkyl cation, on the catalytic activity of soluble and membrane-bound F_1 complex and on the proton conductivity of the F_1–F_0 complex. The results indicate a strong functional cooperative interaction between F_0 and F_1.

Materials and Methods

Chemicals

Oligomycin, valinomycin, and cetyltrimethylammonium bromide were purchased from Sigma (St. Louis, Missouri, USA). Natural nucleotides and enzymes were obtained from Boehringer Mannheim (FRG). All other chemicals were of the highest available degree of purity.

Biological Preparations

Heavy beef-heart mitochondria were prepared as described by Löw and Vallin (1963), stored for 7–30 days at −30°C, and thawed immediately before use. EDTA submitochondrial particles (ESMP) were prepared, as described by Lee and Ernster (1968), by exposing for 60 s heavy beef-heart mitochondria to ultrasonic energy generated by an ultrasonic Branson sonifier (model W 185) with an output of 70 W. Sephadex particles and urea particles (USMP) were prepared from ESMP as described by Racker and Horstmann (1967). F_1 was extracted from ESMP by chloroform as described by Beechey *et al.* (1975). This procedure is particularly convenient because it is easy and rapid; however, it results in a lower activity of the ATPase as compared to other preparations (Beechey *et al.*, 1975). The extracted F_1, when subjected to sodium dodecyl sulfate gel electrophoresis, showed, using

Coomassie blue staining, the characteristic five-band pattern (see also Houstek *et al.*, 1988).

Analytical Procedure

The ATPase activity of F_1 or of submitochondrial particles was determined at 25°C using a regenerating system containing an excess of phosphoenolpyruvate and pyruvate kinase. The released pyruvate was determined spectrophotometrically at 340 nm with an excess of NADH and lactate dehydrogenase (Pansini *et al.*, 1978). Care was taken to ensure a large excess of coupling enzymes. Furthermore, controls with colorimetric determination of pyruvate excluded any effect of amphiphylic cations on the coupling enzymes. The reaction mixture contained, in a final volume of 1 ml: 250 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.4), 1 mM phosphoenolypruvate, ATP (ranging between 0.5 and 2500 μm), 2–5 units of pyruvate kinase, 2.5 units of lactate dehydrogenase, 0.15 mM NADH, and soluble or membrane-bound F_1 (variable protein concentration, as specified in the legend to figures and tables). The reaction was triggered with enzyme or with substrate, as will be mentioned.

For measurements of proton conduction the relaxation of respiratory proton gradient was followed in sonic particles (3 mg protein/ml) incubated in a reaction mixture containing, in a final volume of 1.5 ml, 250 mM sucrose, 30 mM KCl, 0.5 μg valinomycin/mg particle protein, 0.2 mg/ml purified catalase, and 20 mM succinate as respiratory substrate, pH 7.5. Incubation was carried out in a glass vessel, under a constant stream of N_2, at 25°C. Respiration-driven proton translocation was activated by repetitive pulses of 1–3% H_2O_2 (5 μl/ml) (Kopecky *et al.* 1983; Guerrieri *et al.*, 1984). The pH changes, monitored potentiometrically, were converted into proton equivalents by double titration with standard HCl and KOH. Protein concentration was determined by Lowry *et al.* (1951).

Results

Inhibition of Soluble F_1 by Cetyltrimethylammonium

Cetyltrimethylammonium inhibits the ATPase activity of isolated F_1 (Fig. 1A). The inhibitory effect was stronger than that exhibited by octylguanidine, which was previously shown to act on both soluble and membrane-bound F_1 of beef-heart mitochondria (Papa *et al.*, 1975; Tuena de Gomez-Puyou *et al.*, 1977a; Pansini *et al.*, 1978). Cetyltrimethylammonium exerted a pure noncompetitive inhibition on the hydrolytic activity of soluble F_1, and from the Dixon plot, a K_i of 80 μM could be calculated (Fig. 1B). The

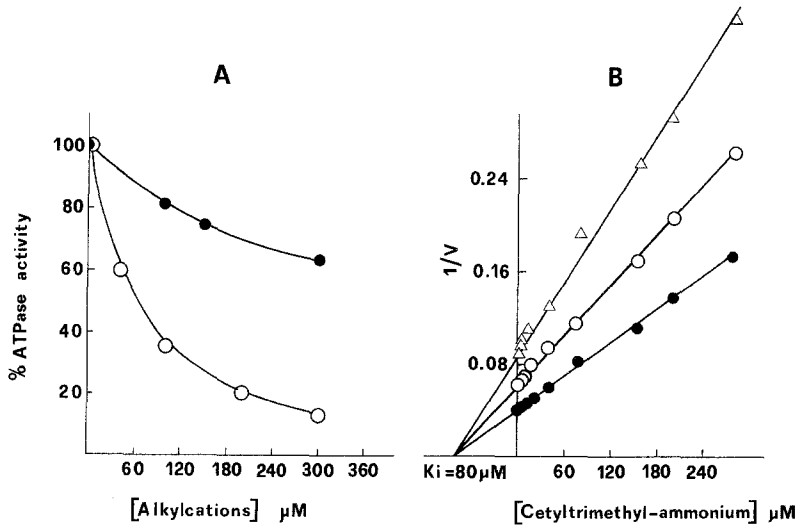

Fig. 1. Inhibition by alkyl cations of ATPase activity in purified soluble F_1 from beef-heart mitochondria. For F_1 purification, see Materials and Methods. For ATPase activity, purified F_1 (5 μg/ml) was added to 1 ml of the reaction mixture described under Materials and Methods. After 2 min incubation the reaction was started by addition of ATP (0.5 mM in Fig. 1A; 0.05–0.3 mM in Fig. 1B) and the oxidation of NADH followed at 340 nm ($\Delta\varepsilon$ mM) = 6.22). (A) The ATPase activity is expressed as percent of the control value (25 μmol ATP hydrolyzed/min · mg protein). Symbols: (●) octylguanidine; (○) cetyltrimethylammonium. (B) Dixon plot for inhibition of F_1 activity by cetyltrimethylammonium. The rate of ATPase activity (V) is expressed in μmol ATP hydrolyzed/min · mg protein. Symbols: (●) ATP 0.3 mM; (○) ATP 0.12 mM; (△) ATP 0.05 mM.

inhibition of the hydrolytic activity of soluble F_1 was found to be reversible upon dilution. In the presence of 200 μM cetyltrimethylammonium the ATPase activity was inhibited by about 87%. A five-fold dilution of the assay mixture with fresh reaction medium containing exactly the same reagents, with the exception of F_1 and cetyltrimethylammonium, decreased the inhibition by cetyltrimethylammonium to only 14%. Controls showed that dilution of the uninhibited enzyme did not affect the ATPase activity. These observations thus show that, in the concentration range used (0–200 μM), cetyltrimethylammonium behaves like a reversible, noncompetitive inhibitor of soluble ATPase.

Inhibition of Membrane-Bound ATPase Activity by Cetyltrimethylammonium

The inhibition of ATPase activity of submitochondrial particles (ESMP) by cetyltrimethylammonium showed a more complex pattern. The inhibition exhibited a sigmoidal dependence on the concentration of

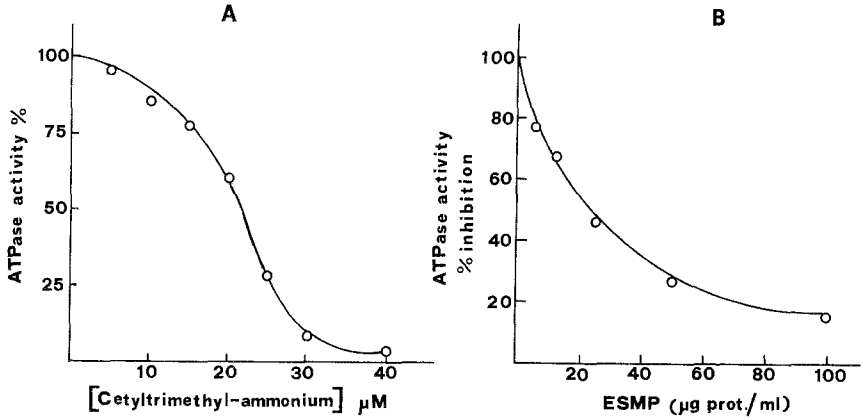

Fig. 2. Inhibition of ATPase activity in ESMP by cetyltrimethylammonium. ESMP were prepared as described under Materials and Methods. (A) ESMP (25 μg/ml) were preincubated 3 min with cetyltrimethylammonium, at the concentrations reported in the figure, in the reaction mixture for determination of ATPase activity, described under Materials and Methods. The activity is expressed as percent of the control ATPase activity (1.2 μmol ATP hydrolyzed/min · mg particle protein). (B) Variable amounts of ESMP (from 6.25 to 100 μg/ml) were preincubated 3 min with 20 μM cetyltrimethylammonium in the reaction mixture described under Materials and Methods, and the ATPase activity was determined as described in the legend to Fig. 2A. The inhibition of ATPase activity is expressed as percent inhibition of the control value (1.2 μmol ATP hydrolyzed/min . mg particle protein).

cetyltrimethylammonium (Fig. 2A). At a single cetyltrimethylammonium concentration, the inhibition of ATPase activity varied from 77 to 15% as the concentration of ESMP was increased from 6 to 100 μg protein/ml.

It should be noted that the inhibition of ATPase activity by cetyltrimethyl ammonium reached the maximum after 1–2 min of preincubation after which no further change occurred. Moreover, the same extent of inhibition was observed if ATP was added before or after cetyltrimethylammonium.

Since cetyltrimethylammonium is a typical alkyl cation, its effects could be due to ionic and/or hydrophobic interactions with components of the H^+-ATPase complex. The effects of glycerol (known to decrease hydrophobic interactions) and of heparin (a polysulfate glycosaminoglycan) were, therefore, examined on the inhibition of ATPase activity of ESMP by cetyltrimethylammonium. As shown in Table I, glycerol (5 and 10% final concentration), which caused *per se* some inhibition of the ATPase activity of the particles, did not affect much the inhibition by cetyltrimethylammonium. On the other hand, heparin largely reversed the inhibitory action of cetyltrimethylammonium (Fig. 3). As expected, heparin, when added before cetyltrimethylammonium, protected ATPase activity against inhibition in a concentration-dependent manner (not shown). It should be mentioned that heparin, at the concentration used in our experiment

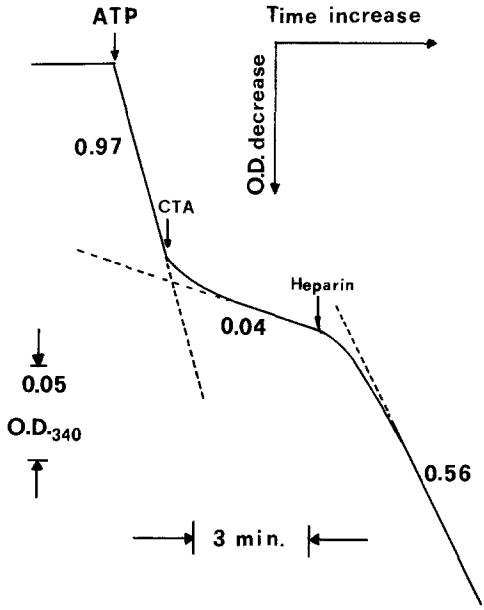

Fig. 3. Reversal by heparin of cetyltrimethylammonium-induced inhibition of ATPase activity in ESMP. For ESMP preparation and ATPase activity determination, see Materials and Methods and legend to Fig. 2A, with the only difference that protein concentration was 12.5 μg/ml. Numbers on the traces indicate the ATPase activity (μmol ATP hydrolyzed/min . mg protein). Where indicated by arrows, cetyltrimethylammonium (15 μM) (CTA) and heparin (5 μg) were added.

Table 1. Effect of Glycerol on ATPase Activity in Sonic Submitochondrial Particles in the Presence and Absence of Cetyltrimethylammonium[a]

Glycerol	Control	ATPase activity (μmol ATP hydrolyzed/min · mg protein) + cetyltrimethylammonium (30 μM)	Inhibition (%)
—	1.20	0.14	88
5	1.06	0.32	69
10	0.78	0.30	61

[a]ESMP were prepared as described under Materials and Methods. For measurement of ATPase activity, see legend to Fig. 2A. Where indicated, glycerol was added 5 min after incubation of ESMP with cetyltrimethylammonium; then the ATPase activity was determined after 5 min incubation.

(5 μg/ml), exerted *per se* only a slight activation (10%) of the ATPase activity.

At fixed particle protein concentration (100 μg/ml) and concentrations of cetyltrimethylammonium from 5 to 50 μM, Dixon plots showed the same noncompetitive pattern as with soluble F_1. A strong upward curvature

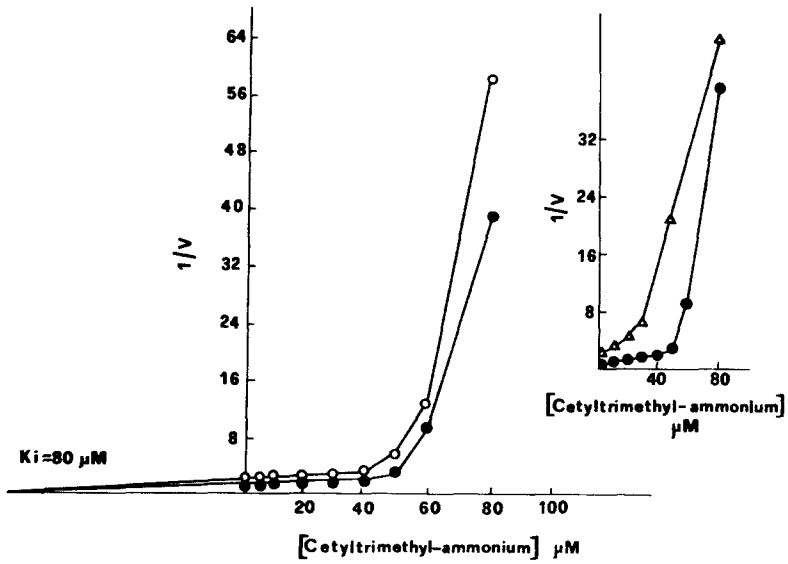

Fig. 4. Dixon plot for inhibition by cetyltrimethylammonium of ATPase activity in ESMP. Additivity with oligomycin. For ESMP preparation, see Materials and Methods. For ATPase activity, ESMP (100 μg/ml) were incubated 3 min with cetyltrimethylammonium (at the concentration reported in the figure); then the ATPase activity was determined as described in the legend to Fig. 2A. Symbols: (●) ATP 0.3 mM; (○) ATP 0.12 mM. In the inset the effect of oligomycin (0.2 μg/mg of protein) (△), added after cetyltrimethylammonium, is shown.

Table II. Effect of Cetyltrimethylammonium on the Apparent K_m for ATP of ATPase Activity in ESMP[a]

Inhibitor (μM)	K_m (mM)
—	0.11
Cetyltrimethylammonium, 40	0.13
Cetyltrimethylammonium, 60	0.15
Cetyltrimethylammonium, 80	0.67
Cetyltrimethylammonium, 150	2.00

[a]For ESMP preparation, see Materials and Methods. For measurement of ATPase activity, ESMP (100 μg/ml) were preincubated with cetyltrimethylammonium (at the concentrations reported in the table) for 3 min; then the reaction was started by the addition of ATP (concentration varied between 0.05 and 1.5 mM) and oxidation of NADH followed at 340 nm. The apparent K_m for ATP was determined by kinetic analysis with Lineweaver–Burk plots.

at inhibitor concentration higher than 50 μM was, however, observed (Fig. 4). At lower concentrations of cetyltrimethylammonium, which did not cause any deviation from linearity in the Dixon plots, the apparent K_m for ATP was practically unaffected by the amphiphylic cation. At concentrations higher than 50 μM the apparent K_m for ATP was significantly enhanced (Table II).

As expected, by lowering the protein concentration from 100 to 25 μg/ml, the concentration of cetyltrymethylammonium required to 'shift' the apparent K_m for ATP from 0.11 mM to 2 mM decreased from 150 to 20 μM (not shown).

Synergistic Effect between Cetyltrimethylammonium and Oligomycin

Figure 5 shows the Dixon plot for oligomycin inhibition of the ATPase activity in ESMP. The pattern obtained was of the linear and noncompetitive type (Fig. 5A). Cetyltrimethylammonium strongly potentiated the inhibition exerted by oligomycin without any derivation from linearity (Fig. 5B). As a consequence, the K_i for inhibition of the ATPase by oligomycin changed from 0.25 to 0.03 μg mg protein.

On the other hand, oligomycin potentiated the inhibition of ATPase activity by cetyltrimethylammonium (see insert to Fig. 4).

Effect of Cetyltrimethylammonium on F_0 Proton Conductivity in Sonic Submitochondrial Particles

In ESMP the anaerobic release of respiratory proton gradient is suppressed by oligomycin and DCCD, indicating that the process takes place through the H^+-ATPase complex (Pansini *et al.*, 1978; Kopecky *et al.*, 1983; Guerrieri *et al.*, 1984). It has been reported that the alkyl cation

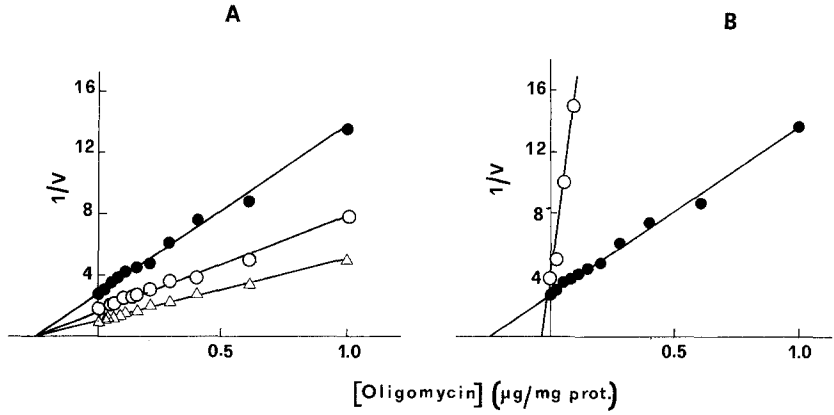

Fig. 5. Dixon plot for inhibition by oligomycin of ATPase activity in ESMP (A). Additivity with cetyltrimethylammonium (B). ESMP were prepared as described under Materials and Methods. For ATPase activity determination: ESMP (100 μg/ml) were preincubated 2 min with oligomycin ($\pm$ 50 μM cetyltrimethylammonium) at the concentration reported in the figure and the ATPase activity determined as described in the legend to Fig. 2A. Symbols: Fig. 5A: (△) ATP 0.3 mM; (○) ATP 0.12 mM; (●) ATP 0.05 mM. Fig. 5B: (●) control with ATP 0.05 mM; (○) + 50 μM cetyltrimethylammonium with 50 μM ATP as substrate.

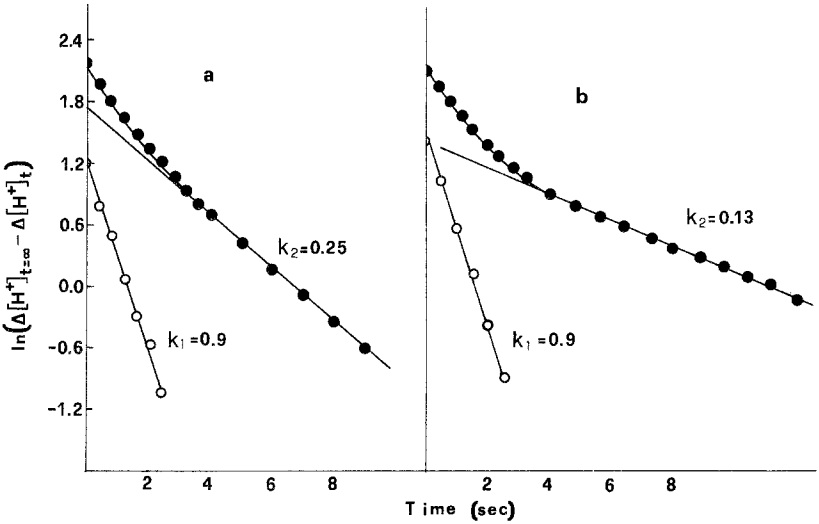

Fig. 6. Double exponential analysis of the kinetics of anaerobic release from ESMP. For ESMP preparation and measurement of proton translocation, see Materials and Methods. Mathematical analysis was carried out as described in Pansini *et al.* (1978). Additions: (a) none; (b) 400 μM cetyltrimethylammonium.

Table III. Effect of Alkyl Cations on Anaerobic Release of Respiratory Proton Gradient in ESMP[a]

Additions	Anaerobic proton release		
	$t_{1/2}$ (sec)	$1/t_{1/2}$ (sec^{-1})	(%)
—	1.18	0.85	
400 μM Guanidine	1.27	0.79	7
400 μM Butylguanidine	1.30	0.77	9
400 μM Octylguanidine	1.56	0.64	25
400 μM Cetyltrimethylammonium	2.00	0.50	41

[a]For ESMP preparation, see under Materials and Methods. Passive proton permeability was measured electrometrically as described under Materials and Methods.

octylguanidine inhibits passive proton conductivity in ESMP (Pansini *et al.*, 1978).

Table III shows that the inhibitory effect by alkyl cations on passive proton conduction in ESMP increased with the lipophilicity of the alkyl cation; i.e., 400 μM guanidine gave only 7% inhibition while 400 μM cetyltrimethylammonium caused 41% inhibition.

Anaerobic release of respiratory proton gradient shows a biphasic pattern that can be resolved in two first-order processes (Fig. 6; see also

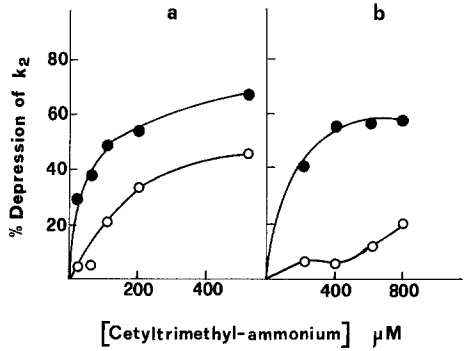

Fig. 7. Effect of cetyltrimethylammonium on slow phase of passive proton conduction in ESMP (a) and in particles deprived of IF_1 (b). Additivity with oligomycin. ESMP (a) and Sephadex particles (b) were prepared as described under Materials and Methods. For determination of the kinetic constant, k_2, of the slow phase of anaerobic H^+ release, see legend to Fig. 6 and Pansini *et al.* (1978). Additions: (○) none; (●) + oligomycin (0.25 μg/mg protein). The data reported in the figure are percent of the control values: (a) 0.25 (○) and 0.15 sec^{-1} (●); (b) 0.6 (○) and 0.4 sec^{-1} (●).

Pansini *et al.*, 1978; Kopecky *et al.*, 1983; Guerrieri *et al.*, 1984). Both are expression of proton conduction by the ATPase complex as judged from their depression by oligomycin or DCCD (Pansini *et al.*, 1978; Kopecky *et al.*, 1983).

Cetyltrimethylammonium, like octylguanidine (see Pansini *et al.*, 1978), inhibited specifically the slow phase (Fig. 6).

Figure 7 shows that the inhibition of the slow phase of passive proton conduction by cetyltrimethylammonium decreased when the ATPase inhibitor protein (IF_1) (Pullman and Monroy, 1963) was removed from ESMP (Fig. 7b). The inhibition of proton conduction by cetyltrimethylammonium was lost in particles deprived of the F_1 sector by urea treatment (not shown).

The presence of the F_0 inhibitor oligomycin strongly potentiated the inhibition of the slow phase of proton conduction by cetyltrimethylammonium in both kinds of particles (Fig. 7).

Discussion

The data reported in this paper show that the alkyl cation cetyltrimethylammonium inhibits the ATPase activity of soluble and membrane-bound F_1. In the case of soluble F_1 the inhibition by alkyl cations increased with their lipophilicity (see also Tuena de Gomez-Puyou *et al.*, 1977b) and the inhibitory pattern was of the pure noncompetitive type. Furthermore, the inhibition by cetyltrimethylammonium could be reversed by dilution,

indicating that it was not due to denaturation or irreversible dissociation of F_1 into its subunits.

The inhibitory action of cetyltrimethylammonium on the ATPase activity of membrane-bound F_1 was synergistic with that exerted by the F_0 inhibitor oligomycin. In particular, it can be noted that cetyltrimethylammonium, which acts on F_1, greatly enhanced the inhibitory potency of oligomycin, the K_i of the latter being lowered by about one order of magnitude. This synergistic effect indicates that conformational changes induced by specific ligands in one sector, F_0 or F_1 respectively, extend over long distances to the other counterpart. This would be in line with the observation that modification of F_0 by DCCD affects the three-site catalytic kinetics in F_1 (Penefsky, 1985).

Cetyltrimethylammonium also inhibited proton conduction by F_0 in sonic submitochondrial particles containing F_1. After release of F_1 by urea treatment of particles, the inhibitory effect of H^+ conduction by cetyltrimethylammonium disappeared.

The inhibition of H^+ conduction was dependent on the lipophilic nature of the alkyl cation and decreased in particles deprived of IF_1 (Sephadex particles). It can be recalled that IF_1 inhibits proton conduction in F_1–F_0 complex probably by regulation of F_0–F_1 interactions (Guerrieri *et al.*, 1987). It is possible that in IF_1-deprived particles the inhibitory action of the F_1 ligand cetyltrimethylammonium on H^+ translocation by F_0 is less effective because F_0–F_1 interactions are impaired (Kozlov and Skulachev, 1977).

The present observations seem, on the other hand, to exclude the possibility that the inhibition of H^+ conduction by alkyl cation is simply due to their binding to the membrane sector (F_0) (Schäfer *et al.*, 1974). The inhibitory action exerted in ESMP by alkyl cations seems to reflect disturbance of F_1–F_0 interactions which may be involved in the control of proton conduction in F_0 and its coupling to the chemical catalysis in F_1.

In conclusion, the inhibitory action of cetyltrimethylammonium, as well as of other amphiphylic cations, results from interaction with negatively charged acidic residues apparently buried in hydrophobic environments of the F_1 moiety.

It is worth nothing that the hydrophobic reagent DCCD inhibits the ATPase activity of F_1 by modification of glutamic residues of the β-subunit of the enzyme (Yoshida *et al.*, 1979, 1981; Matsumo-Yagi and Hatefi, 1984; Tommasino and Capaldi, 1985). It is possible that weak electrostatic interactions of the salt-bridge type between carboxylic and amino groups of residues located in hydrophobic regions are involved in subunit interactions critical for the catalytic and coupling activity of the F_0–F_1 complex.

References

Amzel, L. M., and Pedersen, P. L. (1983). *Annu. Rev. Biochem.* **52**, 801–824.

Beechey, R. B., Hubbard, S. A., Linett, P. E., Mitchell, A. d., and Munn, E. A. (1975). *Eur. J. Biochem.* **148**, 533–537.

Foster, D. L., and Fillingame, R. H. (1982). *J. Biol. Chem.* **257**, 2009–2015.

Godinot, C., and Di Pietro, A. (1986). *Biochimie* **68**, 367–374.

Guerrieri, F., Yagi, A., Yagi, T., and Papa, S. (1984). *J. Bioenerg. Biomembr.* **16**, 251–262.

Guerrieri, F., Scarfò, R., Zanotti, F., Che, Y. W., and Papa, S. (1987). *FEBS Lett.* **213**, 67–72.

Houstek, J., Kopecky, J., Zanotti, F., Guerrieri, F., Jirillo, E., Capozza, G., and Papa, S. (1988). *Eur. J. Biochem.*, in press.

Kopecky, J., Guerrieri, F., and Papa, S. (1983). *Eur. J. Biochem.* **131**, 17–24.

Kozlov, I. A., and Skulachev, V. P. (1977). *Biochim. Biophys. Acta* **463**, 1–27.

Lee, C. P., and Ernster, L. (1968). *Eur. J. Biochem.* **3**, 391–400.

Löw, H., and Vallin, I. (1963). *Biochim. Biophys. Acta* **69**, 361–374.

Lowry, O. H., Rosenbrough, N. J., Fan, A. L., and Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.

Matsumo-Yagi, A., and Hatefi, Y. (1984). *Biochemistry* **23**, 3508–3513.

Norling, B., Hundal, T., Sandri, G., Glaser, E., and Ernster, L. (1984). In *H^+-ATPase ATP Synthase): Structure, Function, Biogenesis. The F_0F_1* Complex of Coupling Membranes (Papa, S., Altendorf, K., Ernster, L., and Packer, L., eds.), ICSU Press, Miami/Adriatica Editrice, Bari, pp. 303–320.

Pansini, A., Guerrieri, F., and Papa, S. (1978). *Eur. J. Biochem.* **92**, 545–551.

Pansini, A., Guerrieri, F., and Papa, S. (1979). In *Membrane Bioenergetics* (Lee, C. P., and Ernster, L., eds.), Addison-Wesley, Reading, massachusetts, pp. 423–428.

Papa, S., Tuena de Gomez-Puyou, M., and Gomez-Puyou, A. (1975). *Eur. J. Biochem.* **55**, 1–8.

Papa, S., Altendorf, K., Ernster, L., and Packer, L. (1984). *In H^+-ATPase (ATP Synthase): Structure, Function, Biogenesis. The F_0F_1 Complex of Coupling Membranes*, ICSU Press, Miami/Adriatica Editrice, Bari, pp. 000–000.

Penefsky, H. S. (1985). *J. Biol. Chem.* **238**, 3762–3769.

Racker, E., and Horstmann, L. L. (1967). *J. Biol. Chem.* **242**, 2547–2551.

Schafër, G., Riegen, E., and Speichert, M. (1974). *Eur. J. Biochem.* **46**, 613–623.

Tommasino, M., and Capaldi, R. A. (1985). *Biochemistry* **24**, 3972–3976.

Tuena de Gomez-Puyou, M., Gomez-Puyou, A., and Salmon, M. (1977a). *Biochim. Biophys. Acta* **461**, 101–108.

Tuena de Gomez-Puyou, M., Gomez-Puyou, A., and Salmon, M. (1977b). *Arch. Biochem. Biophys.* **173**, 326–331.

Vignais, P. V., and Satre, M. (1984). *Mol. Cell. Biochem.* **60**, 33–70.

Yoshida, M., Sone, M., Hirata, H., and Kagawa, Y. (1978). *Biochem. Biophys. Res. Commun.* **84**, 117–122.

Yoshida, M., Poser, J. W., Allison, W., Esch, F. S., and Futai, M. (1981). *J. Biol. Chem.* **256**, 148–153.

Yoshida, M., Allison, W., Esch, F. S., and Futai, M. (1982). *J. Biol. Chem.* **257**, 10033–10037.