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CONTROL OF ACTIVITY STATES OF HEART MITOCHONDRIAL ATPase

ROLE OF THE PROTON-MOTIVE FORCE AND Ca^{2+}

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Summary

The ATPase complex of submitochondrial particles exhibits activity transitions that are controlled by the natural ATPase inhibitor (Gómez-Puyou, A., Tuena de Gómez-Puyou, M. and Ernster, L. (1979) Biochim. Biophys. Acta 547, 252—257). The ATPase of intact heart mitochondria also shows reversible activity transitions; the activation reaction is induced by the establishment of electrochemical gradients, whilst the inactivation reaction is driven by collapse of the gradient. In addition it has been observed that the influx of Ca^{2+} into the mitochondria induces a rapid inactivation of the ATPase; this could be due to the transient collapse of the membrane potential in addition to a favorable effect of Ca^{2+}-ATP on the association of the ATPase inhibitor peptide to F_1-ATPase. This action of Ca^{2+} may explain why mitochondria utilize respiratory energy for the transport of Ca^{2+} in preference to phosphorylation. It is concluded that the mitochondrial ATPase inhibitor protein may exert a fundamental regulatory function in the utilization of electrochemical gradients.

Introduction

A protein that inhibits the hydrolytic activity of soluble and particulate mitochondrial ATPase was isolated by Pullman and Monroy [1]. It was sub-

Abbreviations: EDTA, ethylenediaminetetraacetic acid; F_1-ATPase, soluble, oligomycin-insensitive mitochondrial ATPase; FCCP, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone.

sequently established that the protein inhibited all the ATP dependent reactions of mitochondria [2], and it was proposed that the inhibitor could act as a regulator of ATP synthesis and utilization [2,3].

More recently it was shown that the protein also inhibits the oxidative phosphorylation process of submitochondrial particles [4—6]. In chloroplasts, an almost identical action of the inhibitor has been observed on the photophosphorylation reaction [7]. In view of the possible role of the protein inhibitor on mitochondrial function, it is of importance to note that the experimental data indicated that the prior to the onset of phosphorylation, the ATPase exhibits a transition from an inactive to an active state. This transition was shown to be due to a proton-motive force induced dissociation of the inhibitor from the F_1 component of the mitochondrial ATPase [4,6].

In the light of these findings, several questions arise. One is as to whether the ATPase complex of intact mitochondria exists also in the inactive and active states, and, if so, whether the activity states of the ATPase could regulate the utilization of the proton-motive force for either ATP synthesis and/or some other energy-requiring process, such as ion transport.

In the latter respect, it should be recalled that, under certain conditions, mitochondria have been found to utilize energy derived from electron transport for driving Ca^{2+} influx, in preference to carrying out ADP phosphorylation [8,9].

The results of this work show that the ATPase complex of intact rat-heart mitochondria exists in inactive and active states, and some of the factors that control this transition are described. In addition the experimental findings provide a molecular explanation for the ability of mitochondria to accumulate Ca^{2+} in preference to oxidative phosphorylation.

Material and Methods

Mitochondria from the hearts of 4—6 rats were prepared either by the Nagarse method [9] (the authors are indebted to Dr. A. Vercesi for a practical demonstration of this procedure), or by grinding the minced heart with sand in 0.25 M sucrose, 1 mM EDTA pH 7.4. With respect to the ATPase experiments, both methods gave essentially the same results; however in mitochondria prepared by the Nagarse method, the respiratory control ratios were significantly higher (at least 4 in Nagarse prepared mitochondria and about 2 in the other preparation). Mitochondria were used within 1 h of their preparation.

ATPase activity was measured (unless otherwise stated) in 1.0 ml of an incubation mixture containing 0.8—1 mg mitochondrial protein, 0.125 M sucrose, 12.5 mM Tris-HCl (pH 7.4), and 3 mM ATP. The order of addition of the tested compounds, and other additions are described in the respective tables and figures. Trichloroacetic acid at a final concentration of 6% was added to stop the reaction and inorganic phosphate was determined in the supernatant according to Sumner [10].

Ca^{2+} uptake was determined by monitoring absorbance changes of 30 μM arsenazo (III) at 675—685 nm in a dual wavelength spectrophotometer [11].

Soluble mitochondrial F_1-ATPase was prepared as described elsewhere [12]; the preparation of the ATPase inhibitor protein has also been described [13].

Results

Activation and inactivation of the ATPase by electrochemical gradients

As shown in Table I, the ATPase activity of rat-heart mitochondria is increased several-fold by including the uncoupler FCCP in the incubation mixture. In the presence of glutamate-malate or succinate, the uncoupler stimulated ATP-ase activity becomes higher, whilst in the absence of the uncoupler a lower amount of inorganic phosphate is detected in the presence of the oxidizable substrates; the latter is most probably due to phosphorylation of ADP.

The activation of the uncoupler stimulated ATPase activity by glutamate-malate is inhibited by rotenone and antimycin, and that induced by succinate by antimycin. The stimulation effect of antimycin in the absence of FCCP may be due to an uncoupling effect of this antibiotic.

The aforementioned results indicate that electron transport induces activation of the ATPase complex. The results in Table II indicate that the proton-motive force derived from electron transport is the factor responsible for the activation, since FCCP effectively prevents the activating action of the substrates when added before electron transport is established. This conclusion is in agreement with the studies in Mg-ATP submitochondrial particles, in which it was shown that the ATPase could be activated by a proton-motive force via removal of the endogenous ATPase inhibitor from the F_1 component [4,5,14]. Also it agrees with previous findings of Bertina et al. [15] who used aurovertin as probe of conformational changes of particulate F_1 as induced by the energy state of the particles.

Fig. 1 shows that once the ATPase of intact mitochondria is activated, it remains in that state for a period of at least 7 min. These results indicate that the process is not reversed by collapse of the electrochemical potential, provided the enzyme is maintained in an active hydrolytic state. Moreover the experiment shows that the activity state of the ATPase may be evaluated by assay of

TABLE I

EFFECT OF OXIDIZABLE SUBSTRATES AND RESPIRATORY INHIBITORS OF THE ATPase ACTIVITY OF HEART MITOCHONDRIA

Mitochondria (0.72 mg) prepared by the Nagarse method were incubated in 1 ml of a mixture that contained 0.125 M sucrose, 12.5 mM Tirs-HCl (pH 7.4), and the indicated additions: rotenone 4 μg, antimycin A 0.5 μg, 5 mM glutamate/5 mM malate, or 5 mM succinate. After 3 min, 3 mM ATP ± 1 μM FCCP was added. Hydrolysis was allowed to proceed for 3 min.

Additions	ATPase (μmol P_i formed/mg)	
	—	+FCCP
None	0.09	0.54
Rotenone	0.13	0.35
Antimycin	0.25	0.50
Glutamate/malate	0.04	1.02
Glutamate + rotenone	0.16	0.60
Glutamate + antimycin	0.40	0.55
Succinate + rotenone	0.05	0.97
Succinate + antimycin	0.23	0.52

TABLE II

EFFECT OF THE ORDER OF ADDITION OF SUCCINATE AND FCCP ON THE ATPase ACTIVITY OF MITOCHONDRIA

Mitochondria (0.6 mg) prepared by grinding the tissue with sand were incubated in 1 ml of 0.125 M sucrose, 12.5 M sucrose, 12.5 M Tris-HCl (pH 7.4), 5 μg rotenone, and the indicated additions (A). After 3 min, 3 mM ATP plus the indicated additions in B were included in the mixture. Hydrolysis of ATP was allowed to proceed for 2 min.

Addition A	Addition B	ATPase (μmol P_i formed/mg/min)
5 mM succinate	ATP	0.04
1 μM FCCP	ATP	0.20
5 mM succinate	ATP/FCCP	0.38
1 μM FCCP/5 mM succinate	ATP	0.23

the uncoupler stimulated hydrolytic activity in relatively short times of incubation.

This property of the ATPase may be utilized to study its activity state as influenced by the metabolic condition of the mitochondria. In the experiment of Fig. 2 fresh mitochondria were incubated with glutamate-malate for 2 min, at this time rotenone was added, and this was followed by the sequential additions of succinate and antimycin. Samples of the suspension were withdrawn at pre-determined times and their uncoupler stimulated hydrolytic activity was explored.

The results show that the activity state of the ATPase oscillates in a fashion that is controlled by the build-up and collapse of an electrochemical gradient. Therefore electrochemical gradients would seem to be an important factor in the control of the number of ATPase molecules that exist in the active or

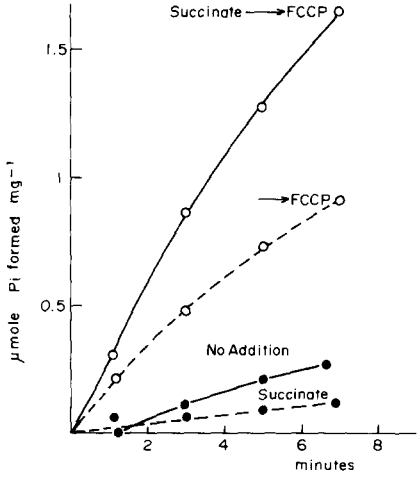

Fig. 1. ATPase activity of heart mitochondria preincubated with succinate. Mitochondria (3 mg) prepared by grinding the hearts with sand were incubated in 5 ml of 0.125 M sucrose, 12.5 mM Tris-HCl (pH 7.4), 4 μg rotenone, and 5 mM succinate as indicated. After 2 min, 3 mM ATP was added with an without 1 μM FCCP as shown by the arrow. At the times indicated aliquots of 1 ml were withdrawn and added to trichloroacetic acid; inorganic phosphate was assayed in the supernatant.

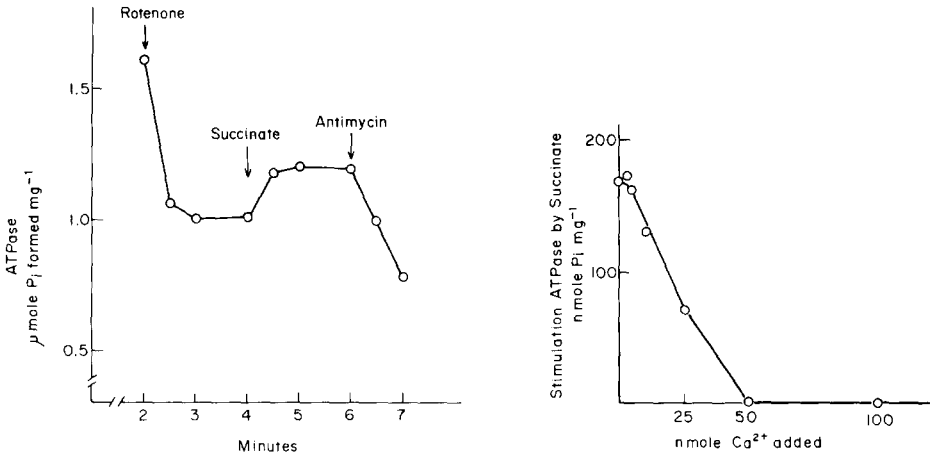

Fig. 2. Changes of ATPase activity of heart mitochondria as regulated by electron flow in the respiratory chain. Mitochondria (3.5 mg) prepared by the Nagarse method were added to 6 ml of incubation medium that contained 0.125 M sucrose, 12.5 mM Tris-HCl (pH 7.4), and 5 mM glutamate/5 mM malate. After two min and the times shown aliquots of 0.5 ml were withdrawn and added to 0.5 ml of 12.5 mM Tris-HCl pH 7.4, 1 μM FCCP and 3 mM ATP (final concentration). The hydrolysis of ATP was allowed to proceed for 3 min. At the arrows and immediately after withdrawl of the aliquot, 5 μg rotenone, 5 mM succinate, and 1 μg antimycin were added to the reaction mixture.

Fig. 3. Effect of Ca^{2+} on the activation of ATPase by succinate. Mitochondria (0.85 mg) prepared by grinding the hearts with sand were incubated in 1 ml of 1.25 M sucrose, 12.5 mM Tris-HCl (pH 8.4), 5 mM succinate, 2 μg rotenone, and the indicated concentrations of Ca^{2+}. After 3 min, 3 mM ATP and 1 μM FCCP were added; hydrolysis proceeded for 2 min. The stimulation of ATPase activity induced by succinate is plotted. Control tubes were incubated without succinate; the inclusion of Ca^{2+}, in the absence of succinate did not modify to a significant extent mitochondrial ATPase.

inactive state. Although the rate of inactivation and activation cannot be precisely determined by this methodology, the 'on' and 'off' rates seem to be compatible with those expected of a regulated process. Indeed in chloroplasts the activation rate of the ATPase via removal of the inhibitor has been shown to be in the millisecond range [7].

Effect of Ca^{2+} on the activity state of the ATPase

Mitochondria from a large variety of cells accumulate Ca^{2+} [16], and it is known that electrochemical gradients are the driving force for Ca^{2+} accumulatin [17]. As the activity state of mitochondrial ATPase seems to be controlled by the proton-motive force (Refs. 4, 5 and 14, and see also Fig. 2), it was considered of interest to explore whether Ca^{2+} transport affected the activity state of the enzyme.

For this purpose mitochondria were incubated with various concentrations of Ca^{2+} and with succinate. After 3 min ATP (+FCCP) was added and the hydrolysis of ATP was measured. Fig. 3 shows that Ca^{2+} effectively prevented the succinate induced activation of the ATPase. Parallel experiments carried out under identical conditions showed that in the presence of succinate, mitochondria could accumulate about 90 nmol of Ca^{2+}/mg in a period of about 1.5 min with 100 nmol Ca^{2+} added per mg of protein (data not shown).

The action of Ca^{2+} in inhibiting the succinate-induced activation could be

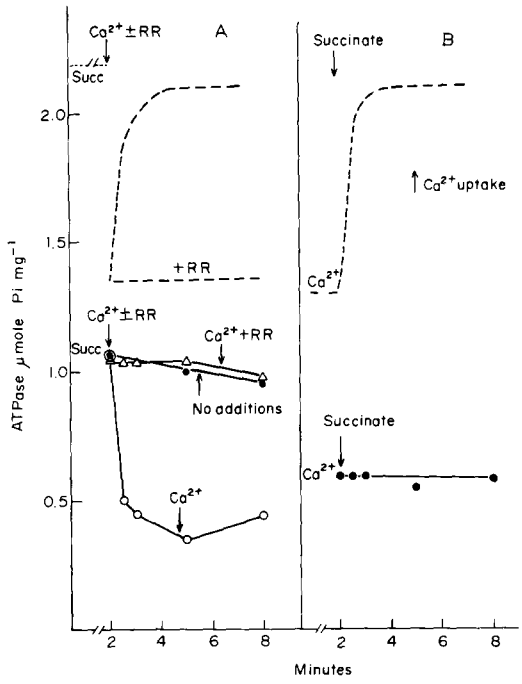

Fig. 4. Inactivation of mitochondrial ATPase by Ca^{2+} mitochondria (3.6 mg) prepared by the Nagarse method were incubated for 2 min in 3 ml of 0.125 M sucrose, 12.5 mM Tris-HCl (pH 7.4), 0.5 mM phosphate, 4 μg rotenone, and either 5 mM succinate (A) or 100 μM Ca^{2+} (B). At the indicated times 0.5 ml of this mixture was added to 0.5 ml Tris-HCl (pH 7.4), 3 mM ATP, and 1 μM FCCP (final concentration) and the hydrolysis measured in a 3-min period. After the first aliquot was withdrawn 100 μM Ca^{2+} was added (open circles in A); in the trace depicted with △———△ 100 μM Ca^{2+} plus 10 nmol ruthenium red (RR) was added, while in the trace illustrated with closed circles (A), no addition was made at 2 min. In B mitochondria were incubated with 100 μM Ca^{2+}, and 5 mM succinate was added at the arrow (2 min). The upper traces (dashed lines) indicate the absorbance changes at 675—685 nm of 60 μM arsenazo (III) of mitochondria incubated in conditions identical to those of the ATPase experiments. In A, Ca^{2+} uptake was started by adding Ca^{2+} (±ruthenium red (RR)) to mitochondria incubated for 2 min with succinate, while in B, uptake was started by adding succinate to mitochondria preincubated with Ca^{2+} for 2 min.

due either to internal Ca^{2+} causing e.g. an inhibition of the activity of adenine nucleotide translocase [18] or a prevention of the activation of the ATPase, due to the utilization of the electrochemical gradient for Ca^{2+} transport.

An action of internal Ca^{2+} on the translocase may be discarded, since the assay of the hydrolytic activity is carried out in the presence of FCCP which induces a release of Ca^{2+} from the mitochondria. Moreover the experiment of Fig. 3 shows that after the enzyme had been activated, the addition of FCCP (+ATP), which induces release of Ca^{2+}, does not reverse the activation. Therefore the results indicate that either internal Ca^{2+} or Ca^{2+} influx, or both may be responsible for inhibiting the activity of the ATPase complex.

To explore the latter alternatives, the experiment of Fig. 4 was carried out. Mitochondria were incubated for two min with succinate, at which time Ca^{2+} was added and the activity state of the ATPase was measured at various time intervals, by assay of the hydrolytic activity of aliquots withdrawn from the incubation mixture. Upon the addition of Ca^{2+} a sudden drop in the activity state of the enzyme was observed. The activity continued to be low for at least

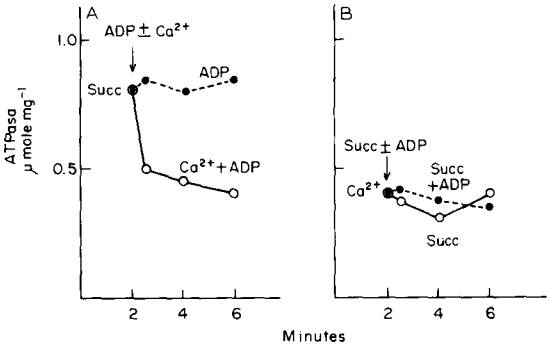

Fig. 5. Activity states of mitochondrial ATPase during state 4-state 3 transitions. Effect of Ca^{2+}. Mitochondria (3.8 mg) were incubated as in Fig. 4. The rest of the experimental conditions were also identical. In A, ADP (1 μmol) was added with and without 100 μM Ca^{2+} as shown to mitochondria preincubated for 2 min with succinate (Succ); whilst in B, 5 mM succinate ± 1 μmol ADP was added to mitochondria preincubated for 2 min with Ca^{2+}. Hydrolysis of the withdrawn samples was allowed to proceed for 2 min.

4 min; it should be mentioned that Ca^{2+} was completely taken up by the mitochondria in a period of about 60 s after its inclusion into the mixture (Fig. 4). The inhibition by Ca^{2+} was prevented by ruthenium red which inhibits the influx of Ca^{2+} into the mitochondria [19,20]. It was also observed that succinate did not activate the ATPase system of mitochondria previously incubated with Ca^{2+} (Fig. 4).

The results of Fig. 4 indicate that the influx of Ca^{2+} into mitochondria either prevents the activation of the ATPase or, alternatively, that it inhibits the enzyme by some other mechanism. In either case, this action of Ca^{2+} could account for the observed ability of mitochondria to accumulate Ca^{2+} in preference to oxidative phosphorylation [8,9].

According to the data of Fig. 5, it would seem that the activity state of the ATPase accounts for this property of mitochondria. Mitochondria incubated for 2 min with succinate show a high ATPase activity, this is not modified during a state 3-state 4 transition, as induced by a limiting amount of ADP (Fig. 5). On the other hand, the simultaneous addition of Ca^{2+} and ADP induces a strong and rapid fall of ATPase activity and the low activity persists for at least 4 min. In agreement with this observation, the findings of Fig. 5 indicate that mitochondria previously exposed to Ca^{2+} (and rotenone), in which the activity is low, the addition of succinate (with and without ADP), does not increase the activity of the ATPase complex (Fig. 5). In other experiments (not shown) it was found that the rate of ATP synthesis was diminished by about 60% after a completed Ca^{2+} cycle; this finding is in agreement with earlier observations by Vercesi et al. [9].

The preceding data indicate that Ca^{2+} influx may regulate the activity state of mitochondrial ATPase. However, the data show that the low activity, as observed in the presence of Ca^{2+}, persists even after Ca^{2+} has been taken up. Therefore it is conceivable that Ca^{2+} per se affects the activity of the ATPase, e.g. by preventing the release of the inhibitor from the enzyme.

The latter possibility was explored by measuring the effect of Ca-ATP on the interaction of soluble purified F_1-ATPase with the ATPase inhibitor. Horst-

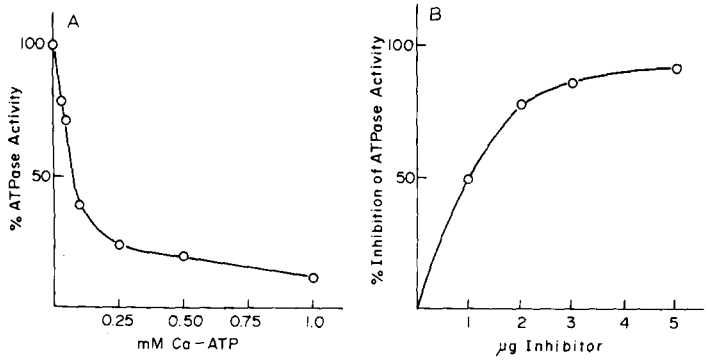

Fig. 6. Effect of Ca-ATP on the interaction of soluble F_1-ATPase with the natural ATPase inhibitor. In A, soluble F_1-ATPase (33 μg) was incubated with 4 μg inhibitor for 10 min in a mixture of 10 mM Tris-Mes (pH 6.5), and the indicated concentrations of Ca-ATP. Aliquots were withdrawn for measurements of ATPase activity. The specific activity of F_1-ATPase was 54 μmol/min/mg. In B, the same incubation conditions were employed except that 0.5 mM Ca-ATP was used, and the indicated concentration of the inhibitor was varied as indicated.

man and Racker [21] have reported that Ca^{2+} promotes the association of F_1 with inhibitor. The data of Fig. 6 show that the association depends on the concentration of Ca^{2+}-ATP added, and that at a concentration of 0.5 mM Ca-ATP the interaction becomes a function of the amount of inhibitor added. In this respect Ca^{2+} behaves similarly to Mg^{2+} (data not shown and Ref. 21).

Discussion

In this work, it is shown that the ATPase complex of intact heart mitochondria may exist in two states, which in reference to previous work [4] may be denoted as inactive and active. The present observations are in agreement with previous findings with particles [4], in which it was shown that the ATPase complex could undergo a reversible transition from the inactive to the active state through the reversible association of the ATPase inhibitor protein of Pullman and Monroy [1] with the F_1 component of the ATPase complex. As the presently described activity transitions of intact mitochondria are strikingly similar to those observed in particles [4], most likely the association and dissociation of the inhibitor from F_1-ATPase is responsible for the changes in activity of the enzyme in intact mitochondria.

The results of this work show that in intact mitochondria, it is possible to determine the activity state of the ATPase complex by measurements of its hydrolytic activity in the presence of an uncoupler. Using this method we observed that the activity state of the enzyme exhibits reversible changes. Although it has not been possible to determine the rates of activation or inactivation, the data suggest that these are compatible with a system that is of metabolic significance. In addition some of the factors or metabolic conditions that control the activity transition have been determined.

In agreement with previous observations in chloroplasts and submitochondrial particles [4—7], the present data indicate that the shift of the enzyme to the active state is mainly, if not solely, controlled by the establishment of electrochemical gradients. On the other hand, the inactivation of the system seems

to be more complex and subject to a larger number of variables. It has been observed that the collapse of the gradient favors the active to inactive transition, but in addition it has been found that the influx of Ca^{2+} also induces inactivation of the enzyme.

We have considered the possibility that the relatively small decrease in the electrochemical potential that occurs during Ca^{2+} influx in heart mitochondria [22] accounts for the inactivation of the ATPase, but alhough the drop in potential may account for the inactivation, other factors are certainly involved. If shifts in electrochemical gradients are responsible for the activity transitions, it would be expected that after Ca^{2+} uptake had taken place, the low activity of the ATPase would change to higher level. This was not the case; after Ca^{2+} had been accumulated the activity of ATPase remained at a low level. This observation suggested that Ca^{2+} may inhibit ATPase directly e.g. by preventing the dissociation of the inhibitor. Indeed we found, in agreement with Horstman and Racker [21], that Ca^{2+} effectively induces the association of the inhibitor with soluble F_1. Therefore it is conceivable that in mitochondria Ca^{2+} has a dual effect; its influx causes an association (or prevents the dissociation) of the inhibitor protein with F_1, due to a decrease in proton-motive force; and internal Ca^{2+} stabilizes, or induces the formation of the F_1-inhibitor complex. In this respect it is worth noting that Carafoli et al. (unpublished results) have observed that the ATP-supported Ca^{2+} uptake by heart and liver mitochondria may be regulated by the activity state of the ATPase.

The experiments described in this work also shed some light on the molecular events that account for the known feature of mitochondria to utilize their electrochemical potential for Ca^{2+} transport in preference to oxidative phosphorylation [8,9]. The inactivation of the ATPase by the inhibitory protein upon Ca^{2+} influx would explain why the proton-motive force is utilized for Ca^{2+} transport in preference to oxidative phosphorylation.

As the inhibitor protein has been isolated from mitochondria from a large variety of cells [1,2,23—27], it is possible that the protein exerts a general and important regulatory role in the overall functionality of mitochondria by controlling the pathways through which the proton-motive force is utilized.

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References

1 Pullman, M.E. and Monroy, G.C. (1963) J. Biol. Chem. 238, 3762—3769
2 Asami, K., Juntti, K. and Ernster, L. (1970) Biochim. Biophys. Acta 205, 307—311
3 Ernster, L., Juntti, K. and Asami, K. (1973) J. Bioenerg. 4, 149—159
4 Gómez-Puyou, A., Tuena de Gómez-Puyou, M. and Ernster, L. (1979) Biochim. Biophys. Acta 547, 252—257
5 Tuena de Gómez-Puyou, M., Nordenbrand, K., Muller, U., Gómez-Puyou, A. and Ernster, L. (1980) Biochim. Biophys. Acta 592, 385—395
6 Harris, D.A., von Tscharner, V. and Radda, G.K. (1979) Biochim. Biophys. Acta 548, 72—84
7 Harris, D.A. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 87—102

8 Jacobus, W.E., Tiozzo, R., Lugli, G., Lehninger, A.L. and Carafoli, E. (1975) J. Biol. Chem. 250, 7863—7870
9 Vercesi, A., Reynafarje, B. and Lehninger, A.L. (1978) J. Biol. Chem. 253, 6379—6385
10 Sumner, J.B. (1944) Science 100, 413—414
11 Scarpa, A. (1976) in Mitochondria: Bioenergetics, Biogenesis, and Membrane Structure (Packer, L. and Gómez-Puyou, A., eds.), pp. 31—46, Academic Press, New York
12 Tuena de Gómez-Puyou, M. and Gómez-Puyou, A. (1977) Arch. Biochem. Biophys. 182, 82—86
13 Kanner, B.E., Serrano, R., Kandrach, M. A. and Racker, E. (1976) Biochem. Biophys. Res. Commun. 69, 1050—1056
14 Van de Stadt, R.J., de Boer, R.L. and van Dam, L. (1973) Biochim. Biophys. Acta 292, 338—349
15 Bertina, R.M., Schrier, P.L. and Slater, E.C. (1973) Biochim. Biophys. Acta 305, 503—518
16 Bygrave, F.L. (1977) Curr. Top. Bioenerg. 6, 259—318
17 Crompton, M. and Heid, I. (1978) Eur. J. Biochem. 91, 599—608
18 Gómez-Puyou, A., Tuena de Gómez-Puyou, M., Klapp, M. and Carafoli, E. (1979) Arch. Biochem. Biophys. 194, 399—404
19 Moore, C.L. (1971) Biochem. Biophys. Res. Commun. 42, 298—305
20 Rossi, C.S., Vasington, F.D. and Carafoli, E. (1973) Biochem. Biophys. Res. Commun. 50, 846—852
21 Horstman, L.L. and Racker, E. (1970) J. Biol. Chem. 45, 1336—1344
22 Nichols, D.G. (1978) Biochem. J. 170, 511—522
23 Pedersen, P.L., Amzel, L.M., Sopen, J.W., Cintrón, N. and Hullihen, J. (1978) in Energy Conservation in Biological Membranes (Schafer, G. and Klingenberg, M., eds.), pp. 159—194, Springer-Verlag, Berlin
24 Satre, M., de Jerphanion, B., Huet, I. and Vignais, P.V. (1975) Biochim. Biophys. Acta 387, 241—255
25 Landry, Y. and Goffeau, A. (1975) Biochim. Biophys. Acta 376, 470—484
26 Chan, H.P. and Barbour, R.L. (1975) Biochim. Biophys. Acta 430, 426—433
27 Ernster, L., Carlsson, C., Hundal, T. and Nordenbrand, K. (1979) Methods Enzymol. 55, 299—407