Plant Physiol. (1989) 91, 487–492
0032-0889/89/91/0487/06/$01.00/0

Received for publication February 9, 1989
and in revised form May 18, 1989

Role of Nonohmicity in the Regulation of Electron Transport in Plant Mitochondria[1]

David G. Whitehouse[2], Anne-Catherine Fricaud, and Anthony L. Moore*

Department of Biochemistry, University of Sussex, Falmer, Brighton, BN1 9QG United Kingdom

ABSTRACT

The relationship between the respiratory rate and the membrane ionic current on the protonmotive force has been investigated in percoll purified potato mitochondria. The dependence of the membrane ionic current on the membrane potential was monitored using a methyltriphenylphosphonium-sensitive electrode and determining the maximal net rate of depolarization following the addition of a respiratory inhibitor. We have confirmed that a nonohmic relationship exists between the ionic conductance and membrane potential. Addition of ATPase inhibitors markedly increased the initial rate of dissipation suggesting that in their absence the dissipation rate induced by respiratory inhibitors is partially offset by H^+-efflux due to the hydrolysis of endogenous ATP. This was corroborated by direct measurement of endogenous ATP levels which decreased significantly following dissipation of the membrane potential. Results are discussed in terms of the regulation of electron transport in plant mitochondria *in vivo*.

It is generally accepted that the free energy which is liberated from the redox reactions catalyzed by the respiratory chain is conserved as a proton electrochemical gradient across the inner mitochondrial membrane (2). The magnitude of this force has been shown in a number of systems to be unrelated to the rate of electron flux since the rate of respiration can be substantially inhibited by malonate or antimycin resulting in only a minor depression of the protonmotive force (6, 8, 21, 24, 27). This has been variously attributed to the nonohmic behavior of the inner membrane (5, 15, 27), to heterogeneity of the coupling of the mitochondria (11), to localized protonic coupling (29), and to variations in the stoichiometry of the redox driven proton pumps (redox slippage) (31). Estimates of the passive conductance of the inner membrane to protons in rat liver mitochondria (5, 15) have shown that conductance does increase under conditions of a high prevailing protonmotive force (imposed as a potassium diffusion potential) in a manner which can quantitatively account for the relationship between oxidation rate and the protonmotive force, and the occurrence of a finite rate of respiration at static head (5).

Although the exact respiratory state of mitochondria in plant cells is uncertain, recent studies suggest that respiration *in vivo* is limited by ADP (10). Unlike their mammalian counterparts, plant mitochondria normally display rapid rates of respiration under ADP limited conditions (9) which has been attributed either to the activity of an endogenous H^+/K^+ antiporter (8, 13) and/or to the operation of nonphosphorylative pathways (22). It has been suggested that nonphosphorylative pathways such as the alternative and rotenone-insensitive pathways constitute a natural slippage (or energy overflow) pathway in plant mitochondria (10, 16) (since they bypass the proton-pumping sites) and, as such, modulation of proton conductance may not be as important as in other tissues.

In contrast to this suggestion there is some evidence in the literature to suggest that proton conductance does regulate respiratory activity. For instance, in isolated mitochondria it has been found that the state 4 rate is generally much slower than the rate of respiration prior to the initial addition of ADP (7) and, furthermore, that the decreased respiratory rate is associated with an increased membrane potential (7, 22). These differences have been attributed to the build-up of endogenous ATP and decreased H^+ conductance through the ATPase (7). However, the mechanism whereby this results in a decreased respiratory rate is ill-understood. In view of these findings, the role of membrane conductance in the regulation of respiration in plant mitochondria warrants further study.

In the present paper we have used a $TPMP^{+}$[3]-sensitive electrode to measure the maximal rate of dissipation of $\Delta\Psi(J_{diss})$ at steady state upon addition of KCN (17) as an indicator of the membrane ionic (H^+) conductance in isolated potato mitochondria. It is assumed that membrane capacitance remains constant during the initial period of measurement and consequently the technique gives a measure of the current flowing across the membrane. Such a technique allows the membrane conductance to be measured without complications due to heterogeneity of coupling and/or redox slippage. It was found that a nonohmic relationship exists between the ionic conductance and membrane potential and furthermore that inhibitors of the F_o/F_1-ATPase considerably increased the maximal rate of dissipation of the membrane potential upon addition of a respiratory inhibitor. Direct measurements of ATP levels revealed that the onset of dissipation was accompanied by a decrease in mitochondrial ATP.

[1] Supported by a grant from the Science and Engineering Research Council.

[2] Present address: Department of Biochemistry and Biology, North East London Polytechnic, London, E15 4LZ, UK.

[3] Abbreviations: $TPMP^+$ methyltriphenylphosphonium; F_1-ATPase, catalytic portion of the ATPase insensitive to oligomycin; F_o-ATPase, membrane sector of the ATPase; $\Delta\Psi$, membrane potential component of the protonmotive force; Δp, protonmotive force; J_{diss}, maximal rate of dissipation of the membrane potential; DCCD, dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone; TPP^+, tetraphenylphosphonium.

The results are discussed in terms of regulation of electron transport of plant mitochondria *in vivo*.

MATERIALS AND METHODS

Materials

Fresh potato tubers (*Solanum tuberosum* L.) were obtained from local sources and stored at room temperature. TPMP bromide was from Aldrich Chemical Co. (Gillingham, Dorset, U.K.). DCCD, venturicidin A, and efrapeptin were kindly donated by Prof. R. B. Beechey (University College of Wales, Aberystwyth, U.K.). Luciferin and luciferase were obtained from LKB (Wallac, Sweden). All other chemicals were from Sigma Chemical Co. (Poole, Dorset, U.K.).

Mitochondrial Isolation

Approximately 2 kg of potato tubers were disrupted by a Waring blendor (7×1 s bursts) at high speed in 1.8 L of ice-cold extraction medium containing 0.3 M mannitol, 10 mM Mops, 1 mM EDTA, 10 mM cysteine, 0.6% (w/v) PVP-40, and 0.1% (w/v) BSA, all adjusted to pH 7.5. The homogenate was filtered through three layers of cheesecloth and centrifuged for 5 min at 350*g*. The supernatant was centrifuged initially at 3,000*g* for 10 min and then at 11,000*g* for 10 min. The pellets were washed in 100 mL of medium containing 0.3 M mannitol, 10 mM Mops, 1 mM EDTA, and 0.1% (w/v) BSA at pH 7.4 and recentrifuged for 10 min at 10,000*g*. The resultant pellets were purified on a continuous Percoll gradient (23) by resuspending in 50 mL of medium containing 0.3 M mannitol, 5 mM Mops, 0.1% (w/v) BSA, and 21% (v/v) Percoll, and centrifuging for 30 min at 22,000*g*. The mitochondrial band was removed and diluted with 40 mL of washing medium and centrifuged for 10 min at 10,000*g*. Final resuspension was in 2 to 3 mL of wash medium to a final concentration of 50 mg/mL. Mitochondria typically displayed respiratory control values of between 3 and 4 with succinate as substrate.

Oxygen Uptake

O_2 consumption was measured polarographically in 2.1 mL reaction medium containing 0.3 M mannitol, 10 mM KH_2PO_4, 1 mM $MgCl_2$, 10 mM KCl, and 10 mM Mops (pH 7.4) in a specially constructed cell housing a Rank oxygen electrode and a $TPMP^+$-sensitive electrode. All experiments were conducted at 20°C.

Membrane Potentials

Continuous monitoring of the membrane potential was achieved using a $TPMP^+$-sensitive electrode, a modification of the TPP^+-sensitive electrode described in (14). The electrode consisted of a platinum wire immersed in 5 mM TPMP bromide and 100 mM KCl in a 400 μL Eppendorf tube separated from the assay medium by a polyvinyl chloride membrane impregnated with tetraphenylboron and was coupled via a salt bridge to a reference electrode. The $TPMP^+$-electrode response was calibrated for each incubation with successive additions of $TPMP^+$ up to a final concentration of 2 to 4 μM and corrected for probe binding as described below. The electrode obeyed the Nernst equation over the range of $TPMP^+$ concentrations used and was unaffected by the inhibitors (or solvents). It should be noted that due to the presence of an endogenous K^+/H^+ antiporter (13), the contribution of the pH component to the protonmotive force is negligible and this was further ensured by the composition of the medium such that $\Delta p = \Delta\Psi$. Membrane potentials were calculated using the Nernst equation on the basis of a measured matrix volume of 1.4 μL/mg protein determined as described (26). To calculate changes of the membrane potential, a correction for the response time of the measuring system was introduced (see below).

$TPMP^+$-Binding Correction

A correction for probe binding was determined by reference to a comparison of [^{3}H]$TPMP^+$ and $^{86}Rb^+$ uptake in mung bean mitochondria measured in parallel incubations. Mitochondria (1 mg/mL) were incubated for 1 min in a medium containing 0.3 M mannitol, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 20 mM HEPES/Tris (pH 7.2) together with either 0.3 μCi/mL of $^{86}Rb^+$ (in the presence of 0.2 μg/mL valinomycin) or 0.5 μCi/mL of [^{3}H] $TPMP^+$. Respiration was initiated by the addition of 1 mM NADH. Mitochondrial membrane potentials were determined from the relevant accumulation ratios following silicone-oil centrifugation as previously described (26). The proportion of lipophilic ion accumulated free to respond to the mitochondrial membrane potential was calculated from the ratio of observed Rb^+ distribution to that of $TPMP^+$. It was found that at the high membrane potential used in the present experiments (*i.e.* 150–220 mV) both $TPMP^+$ and Rb^+ distribution gave the same potential measurements and hence no binding correction had to be introduced. This was only necessary when potentials fell below 150 mV.

$TPMP^+$-Electrode Response Time Correction

This was determined from the time course of the electrode response following the simultaneous addition of 0.5 mM KCN and 0.5 μM FCCP to respiring mitochondria. Dissipation rates measured under these conditions were at least three times faster than our experimental values. As maximal H^+ movement through the inner mitochondrial membrane is assumed under these conditions, it is apparent that the electrode response is both kinetically competent and not rate limiting.

ATP Determinations

Endogenous levels of mitochondrial ATP were determined during the simultaneous measurement of membrane potentials by the addition of 20 μL of 10% (v/v) TCA and 0.2 mM EDTA to 100 μL samples of the assay medium described above. After 10 min extraction the sample was neutralized with 0.1 M NaOH, made up to 900 μl with 0.1 M Tris-acetate, 2 mM EDTA (pH 7.75), and 200 μL ATP Monitoring Reagent (LKB, Finland) added. ATP concentration was measured as light emission in an LKB 1250 Luminometer. After measurement each sample was calibrated internally by spiking with a known amount of ATP.

ATP levels during state 3/4 transitions were determined from 100 μL samples of the assay medium which were treated rapidly with 10 μL of 10 μM oligomycin. The inhibited sample was pipetted into a 400 μL microfuge tube containing 25 μL of 10% (v/v) TCA and 0.2 mM EDTA and 75 μL of silicone oil (AR200/AR20, 10:1 [v/v]). Mitochondria were pelleted through the silicone oil by centrifuging for 60 s in a Beckman Microfuge B. Tubes were frozen at −17°C, cut through the oil layer and the TCA layer neutralized with 0.1 M NaOH. ATP was determined using the luciferin/luciferase system described above. It was found that small amounts of silicone oil (less than 50 μL) did not interfere with the assay system.

Protein was determined as in Lowry *et al.* (19) with BSA as standard.

RESULTS

The membrane ionic conductance was determined from the observed initial rate of dissipation of the membrane potential (J_{diss}) upon addition of a respiratory inhibitor, such as KCN, as outlined (17, 30). Figure 1 shows a typical calibration of the $TPMP^+$ electrode and the generation of a membrane potential upon addition of 5 mM succinate. As indicated in Figure 1, plant mitochondria are relatively deenergized when isolated and hence do not generate any substantial membrane potential in the absence of added substrates. The oxidation of succinate in the absence of ADP routinely generated potentials in excess of 200 mV. The lack of an effect of nigericin on these potentials (not shown) is consistent with the idea that in this particular medium the pH component of the protonmotive force is negligible (22) and, furthermore, serves to emphasize the suitability of this technique as a measure of the ionic conductance across the inner membrane of plant mitochondria. The addition of 0.5 mM KCN results in a rapid dissipation of the membrane potential (2.9 mV/s) and respiratory inhibition. Although there was a delay (0.5–1.0 s) after the addition of KCN before the maximal rate of dissipation was achieved, we assume that the inhibition of respiratory activity was sufficiently fast enough not to have any influence on the dissipation measurements. KCN was chosen as the most suitable inhibitor since the addition of either antimycin or myxothiazol resulted in a slower dissipation rate (approximately 2.0–2.3 mV/s).

In Figure 2 the initial rates of dissipation of the membrane potential and the steady state respiratory rate have been plotted as a function of the membrane potential. Varying membrane potentials were obtained by titration of the respiratory rate with malonate. The graph indicates that a nonohmic rise in the respiratory rate and J_{diss} occurs at high membrane potentials in agreement with data obtained from other systems (6, 21, 24, 27). Although there are other parameters that may be responsible for the nonohmic rise in the respiratory rate (see introduction) in the case of J_{diss}, the result can be satisfactorily explained by changes in the ionic conductance of the inner membrane. The membrane conductance thus increases exponentially at high membrane potentials as originally proposed by Nicholls (24).

According to the chemiosmotic hypothesis, the proton electrochemical gradient is generated by the activity of the respiratory chain and is dissipated either by way of membrane leaks (including the ATPase) and/or substrate translocators (2; and see Fig. 5). It was therefore of interest to determine the effect of inhibitors of the ATPase on the rate of dissipation

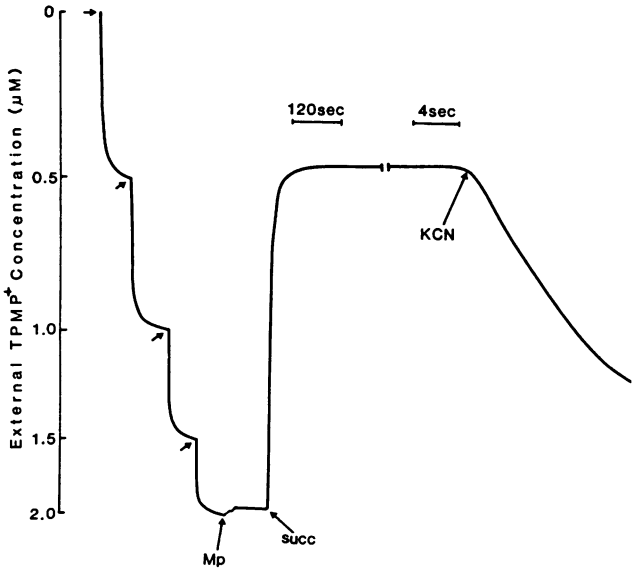

Figure 1. $TPMP^+$ electrode tracing illustrating the generation and dissipation of the membrane potential. Simultaneous measurement of oxygen consumption and steady state membrane potentials were performed in 2.1 mL of reaction medium containing 1 mg mitochondrial protein. The electrode was calibrated with successive additions of 0.5 μM TPMP-bromide before addition of mitochondria. Other additions as indicated: succinate, 5 mM; KCN, 0.5 mM.

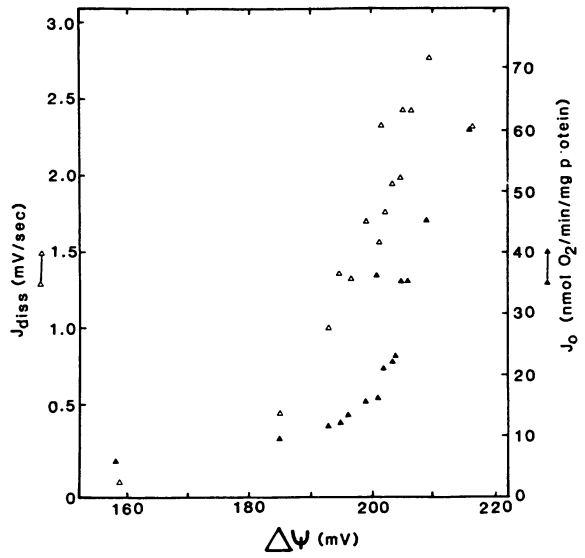

Figure 2. Rate of dissipation and steady state respiratory rate as a function of membrane potential. Mitochondria (1 mg protein) were incubated as in Figure 1 in the presence of 5 mM succinate and 0 to 5 mM malonate and 2 μM TPMP-bromide. Steady state oxygen consumption in the presence of substrate only (J_o:▲) and rate of dissipation of the steady state membrane potential (J_{diss}:△) were followed simultaneously as described in "Materials and Methods." The membrane potential was dissipated by the addition of 0.5 mM KCN.

of the membrane potential under steady state conditions in order to determine the contribution of this route to the overall dissipative pathway and the results are summarized in Table I. In order to minimize H^+ influx via substrate translocators, NADH was used as a respiratory substrate since it is oxidized by a dehydrogenase located on the outer surface of the inner membrane (9). It is uncertain at present as to why dissipation rates with NADH are greater than those with succinate. It can be seen from Table I that inhibition of both the F_o (by DCCD, venturicidin, or oligomycin) and F_1 (by efrapeptin) segments of the ATPase considerably enhances the J_{diss} values elicited by KCN. It should be noted from Table I that the addition of the ATPase inhibitors did not result in an increase in the steady state membrane potential and hence the increased rate of dissipation observed in the presence of these inhibitors cannot be attributed to an enhanced membrane potential. More so such results tend to suggest that the dissipation of the membrane potential (via H^+-influx) by KCN alone is partially offset by a H^+-efflux resulting from the hydrolysis of endogenous ATP.

In order to determine if this was the case, the formation and hydrolysis of ATP was measured quantitatively using the sensitive luciferin/luciferase technique. As can be seen from Figure 3, in the absence of a substrate, the endogenous ATP concentration is approximately 0.55 nmol/mg protein which is comparable to the endogenous levels observed in yeast and mammalian tissues (20, 28). Addition of 5 mM succinate immediately stimulates the formation of ATP to approximately 0.8 nmol/mg protein. The subsequent addition of KCN under these conditions results in a decrease of ATP confirming our suggestion that in the presence of an electron transport inhibitor ATP is hydrolyzed. The high membrane potentials observed under these conditions may result in the release of the ATPase inhibitor protein (IF_1-off) allowing such hydrolysis to occur (18). Presumably inhibition of the ATPase complex prevents this hydrolysis thus enhancing J_{diss}.

In the presence of excess ADP it can be seen from Table I that J_{diss} is decreased presumably due to the elevation of endogenous ATP levels. Measurement of ATP levels confirms this notion as the endogenous ATP concentration rises from approximately 1 nmol/mg protein to approximately 5.5 nmol/mg protein following the addition of 0.1 μM ADP (Fig. 4). It is suggested that the elevated levels of ATP observed under these conditions facilitates further hydrolysis (upon respiratory inhibition) and the concomitant increased H^+-efflux offsets J_{diss} (*i.e.* the rate of H^+-reentry).

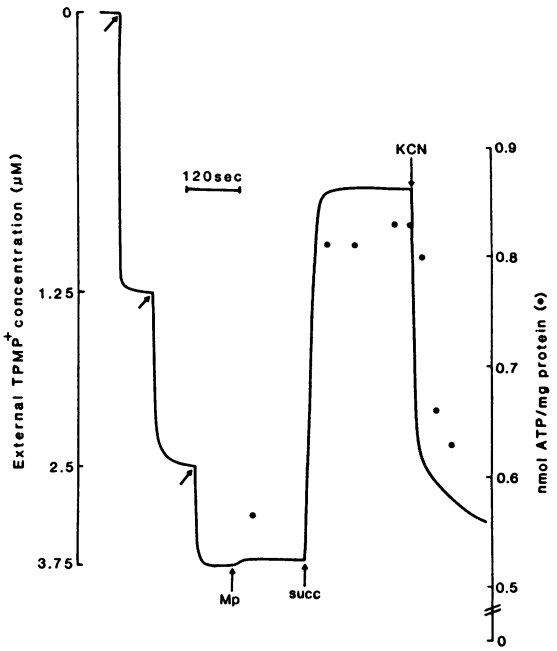

Figure 3. Simulataneous measurements of membrane potential and ATP levels during succinate oxidation. Membrane potentials and ATP levels were measured in 2.1 mL of reaction medium containing 1 mg of mitochondrial protein. The electrode was calibrated by the successive addition of 1.25 μM TPMP-bromide. Respiration was initiated by the addition of 5 mM succinate and following the establishment of a steady state, the membrane potential was dissipated by the addition of 0.5 mM KCN. ATP levels were determined as described in "Materials and Methods" following sampling (200 μL) of the reaction medium at the times indicated.

Table I. *Effect of Energy-Transduction Modulators on the Maximal Rate of Dissipation (J_{diss}) of the Steady State Membrane Potential*

Membrane potentials and maximal dissipation rates were determined in 2.1 mL of reaction medium containing approximately 1 mg of mitochondrial protein. The $TPMP^+$ electrode was calibrated with 2 μM TPMP-bromide. Mitochondria were incubated with either 1.2 μM oligomycin, 10 μM DCCD, 1.4 μM venturicidin A or 1.3 μM efrapeptin for 1 min prior to the addition of 1 mM NADH (all in the absence of ADP). When ADP (100 μM) was added, the dissipation rate is that observed after transition to state 4. In all cases the maximal rate of dissipation of the membrane potential (J_{diss}) was measured after inhibition with 0.5 mM KCN. The data are the means of four separate experiments.

Additions	Membrane Potential	Dissipation Rate
	mV	J_{diss}: *mV/s*
None	212	8.6
ADP	210	5.3
Oligomycin	210	24.9
DCCD	206	16.5
Venturicidin	208	18.3
Efrapeptin	207	16.6

DISCUSSION

In the present paper we have used, similar to others (17, 30), a $TPMP^+$-sensitive electrode to investigate the relationship between the respiratory rate and the membrane ionic conductance with the protonmotive force in plant mitochondria. Experimental conditions were chosen such that the membrane potential component constituted the total protonmotive force thus emphasizing the suitability of J_{diss} as an indicator of the ionic conductance across the inner membrane.

Titration of succinate oxidation with malonate yielded the familiar nonohmic relationship between respiration or ionic-conductance with membrane potential (see introduction). This nonohmic behavior is not in itself surprising having been observed previously in a number of nonplant systems (5, for review) but may have important implications with respect to

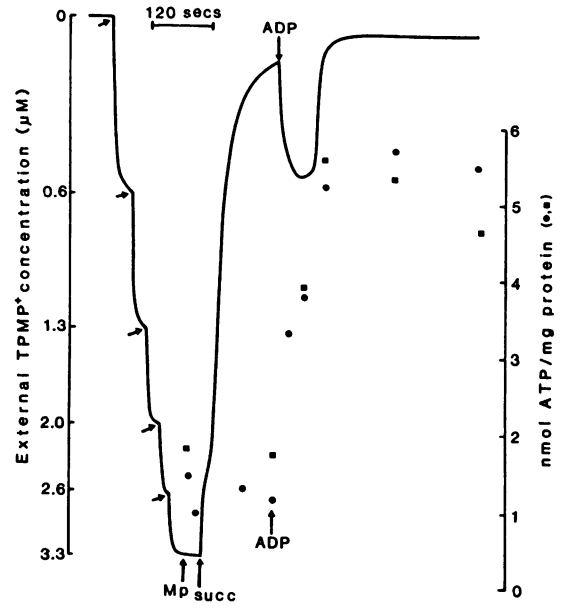

Figure 4. Simultaneous measurements of membrane potential and ATP levels during a state 3/4 transitions, were determined from 100 μL samples treated with 1 μM oligomycin as described in "Materials and Methods."

the regulation of electron transport in plant mitochondria *in vivo*. For instance, plant mitochondria *in vivo* undoubtedly operate with a full complement of TCA-cycle intermediates (10) and not with a single substrate as is the case in this report. If under normal conditions within the plant cell the mitochondrial protonmotive force is high due to adenylate control of the respiratory chain (10, 16) then simultaneous oxidation of additional substrates, such as glycine, can only be accommodated by increases in the protonmotive force. Such increases lead in turn, because of nonohmicity, to a considerable increase in the membrane ionic conductance. Consequently, the results presented in Figure 2 are consistent with a role for membrane conductance in the regulation of electron transport in plant cells *in vivo*. It remains to be determined whether changes in conductance are additional or are alternative to the engagement of nonphosphorylative pathways (such as the alternative oxidase) to cater for respiratory rates with multiple substrates (16). It is well established that state 4 respiration in isolated mammalian mitochondria is controlled by the proton leak rate (4, 12) and this situation appears to exist *in vivo* (4).

By using the sensitive luciferin technique we have been able to measure quantitatively events at very low concentrations of ATP. This has revealed some interesting findings, namely that the addition of a respiratory substrate to deenergized mitochondria results in the increase of ATP even in the absence of added ADP (Fig. 3). Such findings support previous observations and suggestions (1) that plant mitochondria possess a transport mechanism for net adenine nucleotide accumulation apart from the adenine nucleotide translocase that establishes and maintains an intramitochondrial adenine nucleotide pool. The increase in ATP observed in Figure 3 may be due entirely to synthesis; alternatively, it is interesting to speculate that part of this increase may be a result of the protonmotive force being used to release tightly bound ATP from the α-subunit of the ATPase in accordance with an alternating site mechanism for synthesis of ATP (3). In support of this notion, it has recently been shown that energy input on both bovine heart and yeast submitochondrial particles results in the formation of ATP in the absence of added ADP (25). Obviously further work is required to substantiate this suggestion.

Figure 5 depicts the possible routes of dissipation of the membrane potential. These can either be by way of leaks, substrate carriers, or the ATPase. In an attempt to determine if the ATPase can act as a dissipative route, the effect of various ATPase inhibitors was investigated. As indicated in Table I venturicidin, DCCD, and oligomycin, F_o inhibitors and efrapeptin, an F_1 inhibitor, markedly increased J_{diss}. These results can be best explained by proposing that in the absence of these inhibitors dissipation of the ionic conductance is partially offset by an H^+-efflux due to the hydrolysis of endogenous ATP. Direct measurement of endogenous ATP levels (Fig. 3) confirmed that this indeed was the case as levels rapidly decreased upon addition of cyanide. Presumably the increased rate of dissipation observed in the presence of the ATPase inhibitors is due to an inhibition of ATP hydrolysis and resultant decrease in H^+-efflux. Thus, under conditions of respiratory inhibition the ATPase does not act as a route of H^+ reentry. The possibility that ATP hydrolysis occurs in mitochondria under steady state conditions, however, is unlikely since it would have to operate against a high protonmotive force. It is more likely that ATP hydrolysis occurs immediately upon onset of respiratory inhibition in order to buffer the protonmotive force.

Under steady state conditions it has previously been observed that the state 4 respiratory rate is slower than the initial

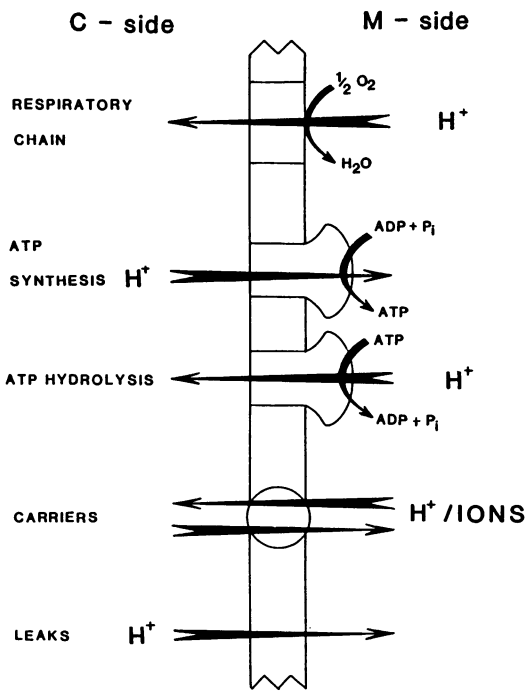

Figure 5. A diagrammatic representation of ion movements across the mitochondrial inner membrane.

rate of respiration (substrate only) and, furthermore, that the addition of ATP or oligomycin during this state decreases the respiratory rate to state 4 levels (7). As can be seen from Figure 3, in the presence of a substrate but absence of ADP, the endogenous ATP levels are low but the membrane potential is high (see also, ref 22). Hence, the ATPase can consititute an ion-influx channel as these conditions result in the release of the ATPase inhibitor protein IF_1 from its inhibitory site on F_1 allowing either ATP synthesis or hydrolysis to proceed (18). A channel therefore exists to dissipate the protonmotive force (either resulting in the phosphorylation of endogenous ADP or slippage) resulting in a concomitant increase in the respiratory rate. In state 4 (following the phosphorylation of ADP) the endogenous ATP levels have increased considerably to approximately 4 mM (Fig. 4). Such concentrations result in an increase in the rate of rebinding of IF_1 to F_1 (19) and consequently the ATPase can no longer act as a route for H^+ reentry. Hence the rate of dissipation of the protonmotive force is decreased with a concomitant reduction of the respiratory rate.

In conclusion, the results presented in this report show that the ionic conductance is variable and dependent upon the value of the membrane potential. Furthermore, this study suggests that the modulation of ionic conductance may play a major role in the regulation of electron transport either as an alternative to or additional to nonphosphorylating pathways, thus facilitating rapid respiratory rates even when under adenylate control.

LITERATURE CITED

1. **Abou-Khalil S, Hanson JB** (1978) Net accumulation of adenine nucleotide by corn mitochondria. *In* G Ducet, C Lance, eds, Plant Mitochondria. Elsevier, Amsterdam, pp 141–150
2. **Boyer PD, Chance B, Ernster L, Mitchell P, Racker E, Slater EC** (1977) Oxidative phosphorylation and photophosphorylation. Annu Rev Biochem **46:** 955–1026
3. **Boyer P, Kohlbrenner WE, Mcintosh DB, Smith LI, O'Neal CC** (1982) ATP and ADP modulations of catalysis by F_1 and Ca^{2+}, Mg^{2+}-ATPases. Ann NY Acad Sci **402:** 65–83
4. **Brand MD, Murphy MP** (1987) Control of electron flux through the respiratory chain in mitochondria and cells. Biol Rev **62:** 141–193
5. **Brown GC, Brand MD** (1986) Changes in permeability to protons and other cations at high protonmotive force in rat liver mitochondria. Biochem J **234:** 75–81
6. **Cotton NPJ, Clarke AJ, Jackson JB** (1984) Changes in membrane ionic conductance, but not changes in slip, can account for the non-linear dependence of the electrochemical proton gradient upon the electron-transport rate in chromatophores. Eur J Biochem **142:** 193–198
7. **Day DA, Wiskich JT, Bryce JH, Dry IB** (1987) Regulation of ADP-limited respiration in isolated plant mitochondria. *In* AL Moore, RB Beechey, eds, Plant Mitochondria—Structural, Functional and Physiological Aspects. Plenum Press, New York, pp 59–66
8. **Diolez P, Moreau F** (1987) Relationships between membrane potential and oxidation rate in potato mitochondria. *In* AL Moore, RB Beechey, eds, Plant Mitochondria—Structural, Functional and Physiological Aspects. Plenum Press, New York, pp 17–26
9. **Douce R** (1985) Mitochondria in Higher Plants. Academic Press, New York
10. **Dry IB, Bryce JH, Wiskich JT** (1987) Regulation of mitochondrial respiration. *In* DD Davies, ed, The Biochemistry of Plants, Vol 11. Academic Press, New York, pp 213–252
11. **Duszynski J, Wojtzak L** (1985) The apparent non-linearity of the relationship between the rate of respiration and the protonmotive force of mitochondria can be explained by heterogeneity of mitochondrial preparations. FEBS Lett **182:** 243–248
12. **Groen AK, Wanders RJA, Wesrehof HV, Van Der Meer R, Tager JM** (1982) Quantification of the contribution of various steps to the control of mitochondrial respiration. J Biol Chem **257:** 2754–2757
13. **Hensley JR, Hanson JB** (1975) Action of valinomycin in uncoupling corn mitochondria. Plant Physiol **56:** 13–18
14. **Kamo N, Muratsugu M, Hongoh R, Kobatake Y** (1979) Membrane potential of mitochondria measured with an electrode sensitive to tetraphenylphosphonium and relationship between proton electrochemical potential and phosphorylation potential in steady state. J Membr Biol **49:** 107–121
15. **Krishnamoorthy G, Hinkle PC** (1984) Nonohmic proton conductance of mitochondria and liposomes. Biochemistry **23:** 1640–1645
16. **Lambers H, Day DA** (1987) Respiration in intact tissues: problems and perspectives. *In* AL Moore, RB Beechey, eds, Plant Mitochondria—Structural, Functional and Physiological Aspects. Plenum Press, New York, pp 321–330
17. **Lemasters JJ, Hackenbrock CR** (1980) The energized state of rat liver mitochondria. J Biol Chem **255:** 5674–5680
18. **Lippe G, Sorgato MC, Harris DA** (1988) The binding and release of the inhibitor protein are governed independently by ATP and membrane potential in ox heart submitochondrial vesicles. Biochim Biophys Acta **933:** 12–21
19. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ** (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193:** 265–275
20. **Lundin M, Pereira da Silva L, Baltscheffsky H** (1987) Energy-dependent formation of free ATP in yeast submitochondrial particles and its stimulation by oligomycin. Biochim Biophys Acta **890:** 279–285
21. **Mandolino G, Desantis A, Melandri BA** (1983) Localised coupling in oxidative phosphorylation by mitochondria from jerusalem artichoke (*Helianthus tuberosus*). Biochem Biophys Acta **723:** 428–439
22. **Moore AL, Rich PR** (1985) Organisation of the respiratory chain and oxidative phosphorylation. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology: Higher Plant Cell Respiration, Vol 18. Springer-Verlag, New York, pp 134–172
23. **Moore AL, Proudlove MO** (1987) Purification of plant mitochondria on silica sol gradients. Methods Enzymol **148:** 415–420
24. **Nicholls DG** (1977) The effective proton conductance of the inner membrane of mitochondria from brown adipose tissue. Dependency on proton electrochemical gradient. Eur J Biochem **77:** 349–356
25. **Penefsky H** (1985) Energy dependent dissociation of ATP from high affinity sites of beef heart mitochondrial adenosine triphosphatase. J Biol Chem **260:** 13735–13741
26. **Proudlove MO, Beechey RB, Moore AL** (1987) Pyruvate transport by thermogenic-tissue mitochondria. Biochem J **247:** 441–447
27. **Sorgato MC, Ferguson SJ** (1979) Variable proton conductance of submitochondrial particles. Biochemistry **18:** 5737–5742
28. **Vandineanu A, Berden JA, Slater EC** (1976) Proteins required for the binding of mitochondrial ATPase to the mitochondrial inner membrane. Biochim Biophys Acta **449:** 468–479
29. **Westerhoff HV, Melandri BA, Venturoli G, Azzone GF, Kell DB** (1984) Mosaic protonic coupling hypothesis for free energy transduction. Biochim Biophys Acta **768:** 257–292
30. **Wojtczak L, Zolkiewska A, Duszynski J** (1986) Energy storage capacity of the mitochondrial protonmotive force. Biochim Biophys Acta **891:** 313–321
31. **Zoratti M, Favroun M, Pietrobon D, Azzone GF** (1986) Intrinsic uncoupling of mitochondrial proton pumps. 1. Non-ohmic conductance cannot account for the nonlinear dependence of static head respiration on $\Delta\Psi$. Biochemistry **25:** 760–769