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Linoleic Acid-induced Activity of Plant Uncoupling Mitochondrial Protein in Purified Tomato Fruit Mitochondria during Resting, Phosphorylating, and Progressively Uncoupled Respiration*

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An uncoupling protein was recently discovered in plant mitochondria and demonstrated to function similarly to the uncoupling protein of brown adipose tissue. In this work, green tomato fruit mitochondria were purified on a self-generating Percoll gradient in the presence of 0.5% bovine serum albumin to deplete mitochondria of endogenous free fatty acids. The uncoupling protein activity was induced by the addition of linoleic acid during the resting state, and in the progressively uncoupled state, as well as during phosphorylating respiration in the presence of benzohydroxamic acid, an inhibitor of the alternative oxidase and with succinate (+ rotenone) as oxidizable substrate. Linoleic acid strongly stimulated the resting respiration in fatty acid-depleted mitochondria but had no effect on phosphorylating respiration, suggesting no activity of the uncoupling protein in this respiratory state. Progressive uncoupling of state 4 respiration decreased the stimulation by linoleic acid. The similar respiratory rates in phosphorylating and fully uncoupled respiration in the presence and absence of linoleic acid suggested that a rate-limiting step on the dehydrogenase side of the respiratory chain was responsible for the insensitivity of phosphorylating respiration to linoleic acid. Indeed, the ADP/O ratio determined by ADP/O pulse method was decreased by linoleic acid, indicating that uncoupling protein was active during phosphorylating respiration and was able to divert energy from oxidative phosphorylation. Moreover, the respiration rates appeared to be determined by membrane potential independently of the presence of linoleic acid, indicating that linoleic acid-induced stimulation of respiration is due to a pure protonophoric activity without any direct effect on the electron transport chain.

Since the advent of Mitchell's chemiosmotic theory for energy conservation in 1961 (1), it has been recognized that the proton electrochemical gradient ($\Delta\mu H^+$)[1] generated across the inner mitochondrial membrane by the respiratory chain is used not only to phosphorylate ADP but also to support other processes such as the transport of inorganic cations (2) and various anionic metabolites (3), the nucleotide transhydrogenase reaction (4), and the electrophoretic exchange of ATP^{4-}/ADP^{3-} (5).

Energy dissipation in plant mitochondria can be mediated by two processes: a redox potential dissipating system, the quinol alternative oxidase (AOX) branched from the main respiratory chain at the level of ubiquinone (6, 7); and a proton electrochemical potential dissipating system, the plant uncoupling mitochondrial protein (PUMP) (8, 9). PUMP functions similarly to the uncoupling protein of mammalian brown adipose tissue which exports anionic free fatty acids (FFA) from the mitochondria, which can subsequently return in a protonated form across the membrane phospholipid bilayer through a "flip-flop" mechanism (10). Because for each cycle of FFA transport one H^+ remains in the matrix (11), there is an increase in proton conductance through the mitochondrial inner membrane, thereby decreasing $\Delta\mu H^+$ and energy availability for energy-consuming processes. AOX catalyzes quinol-oxygen oxyreduction that is not linked to proton pumping outside the matrix. As a result, no $\Delta\mu H^+$ is built and redox free energy is not conserved. AOX is not sensitive to cytochrome pathway inhibitors such as cyanide, antimycin, or myxothiazol but is inhibited by hydroxamic acids such benzohydroxamate (BHAM). Both the PUMP and AOX activities dissipate free energy into heat at the expense of a H^+ gradient potential and of a redox potential, respectively, thereby potentially decreasing the yield of ATP production.

The two energy-dissipating systems could play a role in thermogenesis, as is obvious for AOX activity in plant thermogenetic tissues (spadices of Araceae) which leads to temperature rise involved in reproductive processes (12). An increase in temperature is also observed during the ripening of climacteric fruits and is attributed to the stimulation of AOX activity (13, 14). However, in nonthermogenic tissues or cells, PUMP and AOX must play a more fundamental role at the level of energy balance of the cell. Indeed, the supply of reducing substrates

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[1] The abbreviations used are: $\Delta\mu H^+$, proton electrochemical gradient; AOX, alternative oxidase; PUMP, plant uncoupling mitochondrial protein; FFA, free fatty acids; BHAM, benzohydroxamic acid; LA, linoleic acid; $\Delta\Psi$, mitochondrial transmembrane electrical potential; BSA, bovine serum albumin; TPP^+, tetraphenyl phosphonium; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; olig, oligomycin; V, respiratory rate; V4, state 4 respiration; V3, state 3 respiration.

and the energy and carbon demand for biosynthesis are coupled by respiration. Metabolic conditions that lead to a high reducing power or a high phosphate potential reflect an imbalance between the supply and demand processes and result in a decrease in electron flux through the cytochrome pathway. The modification of AOX activity, which is not controlled by the energy status of the cell and is finely tuned (allosteric and redox status of the protein and expression) (6), could counteract these imbalances. On the other hand, PUMP control through allosteric inhibition by purine nucleotides and the FFA availability is well recognized (9). The uniport of FFA (*e.g.* linoleic acid, LA) through PUMP is driven by the mitochondrial transmembrane electrical potential ($\Delta\Psi$). Therefore, PUMP activity could also counteract the imbalances described above mainly by decreasing $\Delta\Psi$. The possible connection between the regulation of the AOX and PUMP energy-dissipating systems and their possible complementarity could be of utmost importance for the organelle oxidative phosphorylation efficiency and for the energy status of the cell.

In this work, we have used FFA-depleted mitochondria of green mature tomato fruits, *Lycopersicon esculentum*, a climacteric fruit known to express the PUMP and AOX[2] proteins. We have investigated whether LA-induced stimulation of respiration reflects pure uncoupling and whether LA-induced PUMP activity is able to divert energy from oxidative phosphorylation, *i.e.* if PUMP is actually active during state 3 respiration.

MATERIALS AND METHODS

Plant Material—Tomato plants (*L. esculentum*) were grown in a greenhouse at the Centro de Biologia Molecular e Engenharia Genética. Tomato fruits were harvested at a nearly developed stage (but still completely green) and were used the same day.

Isolation of Tomato Mitochondria—Usually, 0.5 kg of tomatoes was sliced and homogenized in a domestic blender after removal of the seeds. The juice obtained was immediately diluted to a final volume of 400 ml with a medium containing 500 mM sucrose, 0.2 mM EGTA, 4 mM cysteine, and 40 mM Hepes, pH 7.8. During homogenization, the pH was kept between 7.2 and 7.8 by adding 1 N KOH. After filtration of the homogenate through a layer of polyester cloth, crude mitochondria were subsequently isolated by a conventional differential centrifugation ($500 \times g$ for 10 min, $12{,}300 \times g$ for 10 min) and then washed twice in a medium containing 250 mM sucrose, 0.3 mM EGTA, and 10 mM Hepes, pH 7.2.

Mitochondria were subsequently purified on a 21% self-generating Percoll gradient by a method modified from Van den Berger *et al.* (15). The gradient medium contained 21% (v/v) Percoll, 250 mM sucrose, 0.3 mM EGTA, 10 mM Hepes, pH 7.2, and 0.5% (w/v) bovine serum albumin (BSA). The presence of BSA in the medium allowed chelating of FFA from the mitochondrial suspension. The gradient was centrifuged at $40{,}000 \times g$ for 30 min. Mitochondria were collected and washed three times in 250 mM sucrose, 0.3 mM EGTA, and 10 mM Hepes, pH 7.2. Mitochondrial protein concentration was determined by the biuret method (16).

Assay Procedures—Oxygen consumption was measured with a Clark-type electrode (Yellow Springs Instruments Co.) in 1.3 ml of standard incubation medium (25 °C) containing: 125 mM sucrose; 65 mM KCl; 10 mM HEPES, pH 7.4; 0.33 mM EGTA; 1 mM $MgCl_2$; and 2.5 mM KH_2PO_4, with 0.4–0.5 mg of mitochondrial protein. All measurements were made in the presence of 10 mM succinate, 1.5 mM BHAM, 170 μM ATP, and 5 μM rotenone.

The mitochondrial transmembrane electrical potential was measured under the same conditions as oxygen uptake, except that the standard incubation medium additionally contained 3 μM tetraphenylphosphonium (TPP^+), and a TPP^+-selective electrode was used as described by Kamo *et al.* (17). For calculation of the $\Delta\psi$ value, the matrix volume of tomato mitochondria was assumed as 2.0 μl/mg of protein. The membrane potential was calculated assuming that the TPP^+ distribution between mitochondria and medium followed Nernst's equation. Corrections were made for the binding of TPP^+ to mitochondrial membranes as described by Jensen *et al.* (18).

[2] A. M. Almeida, W. Jarmuszkiewicz, H., Khomasi, P. Arruda, A. E. Vercesi, and F. Sluse, unpublished data.

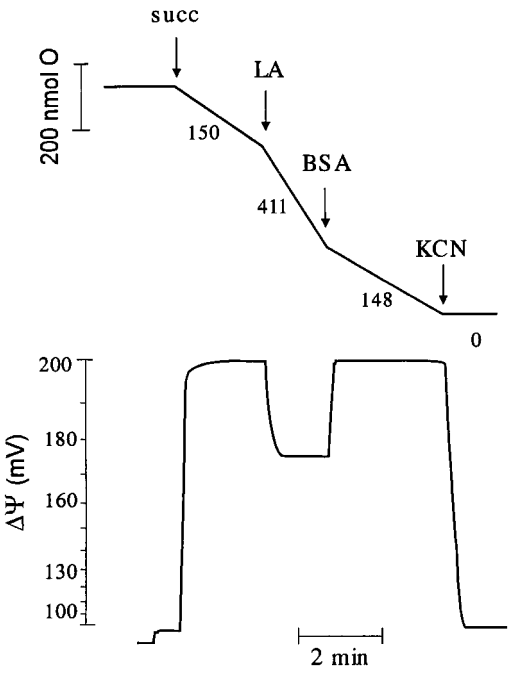

FIG. 1. **Effect of LA on respiration rate and transmembrane potential ($\Delta\Psi$) of green tomato mitochondria depleted of free fatty acids.** Assay conditions as described under "Materials and Methods." Mitochondria were incubated in the presence of 1.5 mM BHAM, 170 μM ATP, 5 μM rotenone, and 2.5 μg/mg protein oligomycin. 10 mM succinate (succ), 10 μM LA, 0.5% BSA, and 1.5 mM KCN were added where indicated. Numbers on the trace refer to O_2 consumption rates in nmol of oxygen atom/min/mg of protein. The membrane potential changes are shown in mV.

The ADP/O ratio was determined by an ADP pulse method with succinate (+ rotenone) as oxidizable substrate. The total amount of oxygen consumed during state 3 respiration was used for calculation of the ratio.

Chemicals—All chemicals were purchased from Sigma.

RESULTS

Respiration Rate and Membrane Potential Measurements in Green Tomato Fruit Mitochondria—Fig. 1 shows the oxygen consumption and parallel $\Delta\Psi$ measurements with succinate (+ rotenone) in the presence of BHAM in green tomato mitochondria depleted of FFA by isolation and purification in the presence of BSA. An increase in state 4 respiration from 151 to 411 nmol of oxygen atom/min/mg of mitochondrial protein was observed after the addition of 10 μM LA. This acceleration in respiration was accompanied by a drop in $\Delta\Psi$ from 200 to 176 mV which was related to the induction of PUMP activity by LA. The original respiratory rate and initial $\Delta\Psi$ value could be reestablished by adding 0.5% BSA which chelates LA. The similar oxygen uptake before the addition of LA and after the addition of BSA confirmed the absence of endogenous FFA in the mitochondrial suspension. Indeed, if residual FFA had been present, the oxygen consumption rate after the addition of BSA would have been smaller than the initial state 4 respiration because of the suppression of endogenous FFA-sustained PUMP activity. The subsequent addition of cyanide blocked the respiration and collapsed the $\Delta\Psi$. No residual respiration was observed in the presence of both BHAM and cyanide.

Correlation between the Mitochondrial Respiratory States and LA-induced Respiration in the Presence of BHAM—The relationship between the mitochondrial respiratory state and LA-induced PUMP stimulation of respiration was studied by the addition of LA in the presence or absence of either carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), oligomycin (olig), or ADP (Fig. 2).

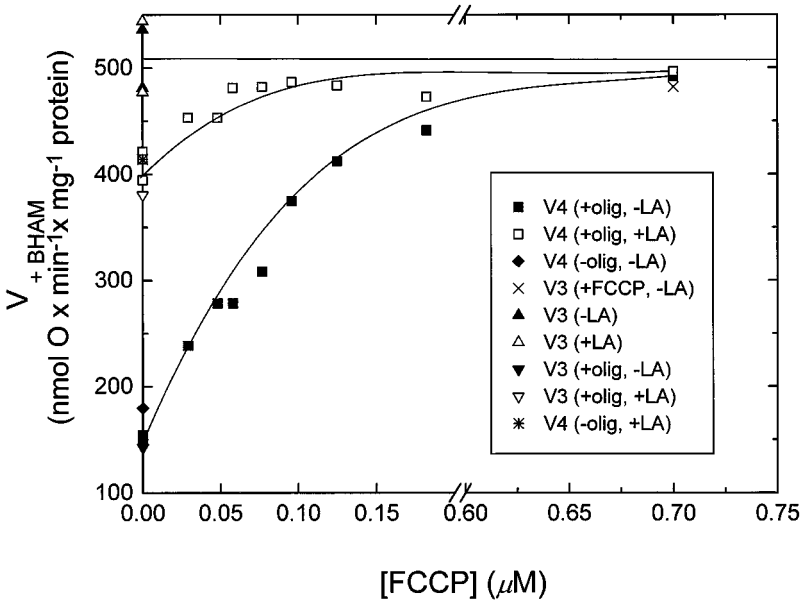

FIG. 2. **Influence of FCCP concentration on the oxygen uptake in various states of respiration in the presence and absence of linoleic acid.** Assay conditions as described under "Materials and Methods." All measurements were made in the presence of 10 mM succinate, 5 μM rotenone, 1.5 mM BHAM, and 170 μM ATP. State 3 respiration was reached by the addition of 2 mM ADP. The concentration of oligomycin used (where indicated) was 2.5 μg/mg of mitochondrial protein. Respiration (in the absence or presence of 10 μM LA) was decreased progressively with increasing concentrations of FCCP (0–0.7 μM). Respiration rates in the presence of BHAM (V_{+BHAM}) are in nmol of oxygen atom/min/mg of protein. The respiration conditions of state 4 (V4) and state 3 (V3) with corresponding symbols are shown in the legend box. *Horizontal line*, mean value of V3 ± LA.

A state 4 respiration rate of 180 nmol of oxygen atom/min/mg of protein (Fig. 2, ♦) and state 3 respiration rates of 536 and 481 nmol of oxygen atom/min/mg of protein (Fig. 2, ▲) confirmed the good coupling of mitochondria (respiratory control factor = 2.8). Both states 3 and 4 were inhibited by olig to rates of 140–150 nmol of oxygen atom/min/mg of protein (Fig. 2, ▼ and ■, respectively on the *Y* axis). In contrast, only state 4 was stimulated by 0.7 μMFCCP (from 180 to 492 nmol of oxygen atom/min/mg of protein, not shown). The uncoupled respiratory control (respiratory rate in the presence of FCCP *versus* respiratory rate in the presence of olig, V_{+olig}) was 3.5, again indicating the good coupling. The addition of 10 μM LA during steady-state state 4_{+olig} respiration, in the presence of increasing concentrations of FCCP, stimulated the respiratory rate (Fig. 2, □ *versus* ■). However, the stimulation decreased progressively with increasing FCCP concentrations and disappeared in the fully uncoupled state (at 0.7 μM FCCP). Moreover, 10 μM LA did not stimulate steady-state state 3 respiration (Fig. 2, Δ *versus* ▲) as well as fully uncoupled state (Fig. 2, ×). The LA-induced stimulation of state 4 respiration could be attributed to the activation of PUMP that increased the proton conductance of the membrane and consequently increased H^+ reentry into the matrix. The absence of LA stimulation in state 3 and in a fully uncoupled state could be due to inadequate $\Delta\Psi$, which could not allow PUMP activation, or more likely due to maximal electron flux reached in both state 3 and uncoupled state.

Absence of LA-induced Respiration in State 3 and Phosphorylation Efficiency—The absence of stimulation of state 3 respiration by LA could suggest that PUMP was not stimulated by LA during phosphorylating respiration. If this was the case, the phosphorylation efficiency would not be modified by LA and would yield the same ADP/O ratio in the presence and absence of LA. Table I shows the ADP/O ratio determined during an ADP pulse (with AOX blocked by BHAM) in the absence of BSA and GTP, and in the presence of LA (0, 3.9, and 8 μM) with succinate (+ rotenone) as the oxidizable substrate. A LA concentration-dependent decrease in the ADP/O ratio was observed, indicating a decrease in oxidative phosphorylation efficiency with increasing LA concentration. Thus, the absence of stimulation of state 3 respiration by LA was nevertheless accompanied by an activation of PUMP by LA that divert energy from oxidative phosphorylation.

Voltage Dependence of Electron Flux in the Respiratory Chain—Couples of measurements ($\Delta\Psi$, rate of O_2 consumption) were plotted in Fig. 3 for various conditions where $\Delta\Psi$ was modified 1) by olig in state 4 (■), 2) by increasing LA concentrations in state 4_{+olig} (●), 3) by addition of saturating ADP (state 3) plus LA (▲) or minus LA (Δ), and 4) by increasing concentrations of FCCP in state 4_{+olig} in the presence of 10 μM LA (○). The maximal respiration in uncoupled state (0.7 μM FCCP) was 495 nmol of oxygen atom/min/mg of protein. The mean value of respiratory rate in state $4_{\pm olig}$ was 170 ± 16 nmol of oxygen atom/min/mg of protein (S.D., n = 8), and the corresponding mean $\Delta\Psi$ value was 200 ± 2 mV (S.D., n = 8). The state $3_{\pm LA}$ respiratory rate (at LA concentration 3.9, 6, or 10 μM) was 501 ± 10 nmol of oxygen atom/min/mg of protein (S.D., n = 7) and the mean $\Delta\Psi$ value was 174 ± 2 mV (S.D., n = 7). A set of conditions constituted a single force-flow relationship: state $4_{\pm olig}$ (□, ■), state 4_{+olig} with increasing concentrations of LA (●), and state $3_{\pm LA}$ at various concentrations of LA (Δ, ▲). This single relationship meant that LA did not interact with the electron transport chain with succinate (+ rotenone) as oxidizable substrate and had a pure protonophoric effect. Moreover, the addition of increasing concentrations of FCCP to state 4_{+olig} plus 10 μM LA (Fig. 3, ○) showed clearly that maximal electron flux was reached as proposed above.

TABLE I
Influence of linoleic acid concentration added to free fatty acid-depleted mitochondria on the ADP/O ratio with succinate (+ rotenone) as oxidizable substrate

Assay conditions as described under "Materials and Methods."

	ADP/O		
	No LA (n = 8)	+3.9 μM LA (n = 6)	+8 μM LA (n = 4)
Av.	1.303	1.074	0.735
S.D.	0.031	0.050	0.059
S.E.M.	0.011	0.021	0.030

DISCUSSION

The demonstration by Garlid *et al.* (11) that anionic fatty acids are the transported substrates of uncoupling protein and that this electrophoretic efflux of univalent amphiphilic anions coupled with a fast return of protonated fatty acids across the phospholipid bilayer results in mitochondrial uncoupling has provided the mechanistic basis for understanding of the carrier-mediated uncoupling by the H^+ circuit through uncoupling

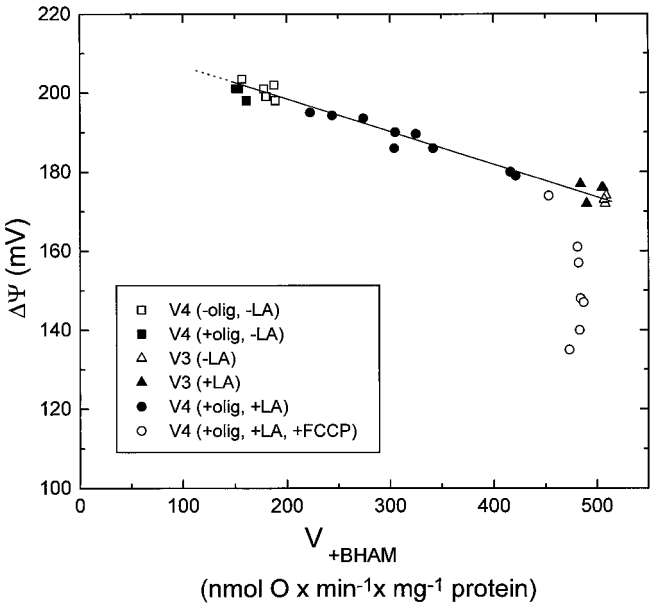

FIG. 3. **Relation between ΔΨ and rate of mitochondrial respiration in the presence of BHAM.** Assay conditions as described under "Materials and Methods." All measurements were made in the presence of 10 mM succinate, 5 μM rotenone, 1.5 mM BHAM, and 170 μM ATP. State 3 plus (▲) and minus (Δ) LA was reached by the addition of 2 mM ADP. □, state 4_{-olig}; ■, state 4_{+olig} (concentration of olig was 2.5 μg/mg of mitochondrial protein); ○, state 4_{+olig} plus 10 μM LA with increasing concentrations of FCCP (0.03–0.7 μM); ●, state 4_{+olig} with increasing concentrations of LA (0.8–10 μM). Respiratory rates (V_{+BHAM}) are expressed in nmol of oxygen atom/min/mg of protein. Membrane potential values (ΔΨ) are presented in mV.

protein. Such fatty acid cycling can increase the mitochondrial respiration in the absence of energy-requiring processes. Since PUMP possesses transport properties similar to those of the uncoupling protein of brown adipose tissue mitochondria, linoleic acid, a naturally abundant plant FFA, strongly activates the H^+ transport and induces a membrane potential drop in potato tuber mitochondria respiring with succinate in state 4 (19). The dependence of FFA-induced H^+ transport on ΔΨ has been documented in proteoliposomes containing PUMP in which there is an exponential increase in H^+ transport with increasing applied voltage (K^+ diffusion potential) (20).

We have shown here that the addition of 10 μM LA to FFA-depleted green tomato mitochondria in the resting state (+ olig, + BHAM) stimulated respiration (+172%) and caused a drop of 25 mV (−12.5%) in the ΔΨ (Fig. 1). This increase in respiration linked to PUMP activation by 10 μM LA (2.7×) is comparable to the increase in 10 μM LA-induced H^+ transport detected by passive osmotic swelling in the presence of 2 mM Mg^{2+} in potato tuber mitochondria and is close to the half-maximum effect of LA in these mitochondria (19).

At concentrations above 40 μM, LA by itself caused swelling of potato tuber mitochondria in suspension containing 0.2 mg of protein/ml of incubation medium (19). Respiratory experiments in green tomato mitochondria showed that already 20 μM LA decreased both state 3 and LA-induced state 4 respiration with 0.3 mg of protein mitochondrial/ml of incubation medium (data not shown). It seems that the concentration of LA causing a half-maximum effect on respiration in intact mitochondria was much lower than the apparent K_m determined in reconstituted systems (83 μM) (20). In tomato mitochondria, the small drop in ΔΨ (from 200 mV to 175 mV) (Fig. 1) caused by 10 μM LA in state 4 respiration, with both ATPase and AOX activities blocked, resulted in a respiration rate equal to 80% of the fully uncoupled state with FCCP. This drop in ΔΨ cannot be compared with membrane potential changes during LA-induced uncoupling of potato tuber mitochondria respiring with succinate since the latter were not quantified (19).

A striking observation was the absence of LA stimulation of state 3 respiration (Fig. 2). The presence of ADP was not responsible for the lack of LA-induced respiration since stimulation by LA was also seen when state 3 was inhibited by olig (Fig. 2). State 3 respiration (±10 μM LA) was found to be equal to state 4 + 0.7 μM FCCP (±10 μM LA) and to state 3 + 0.7 μM FCCP (Fig. 2). This suggests that the respiratory chain was saturated (*i.e.* working at maximal rate), with the limiting step likely being the succinate dehydrogenase (complex II). Such a situation would not permit LA-induced acceleration of state 3 respiration. This does not mean that PUMP activity was not induced in state 3 respiration. Indeed, maximal electron flux in respiratory chain building $\Delta\mu H^+$, which is used for ATP synthesis in the absence of LA, could be shared between oxidative phosphorylation and PUMP activity in the presence of LA. Measurements of ADP/O ratio have been used successfully to determine the contribution of a dissipating pathway (AOX) in amoeba mitochondria during state 3 respiration (21). This approach could provide information about a possible participation of PUMP in state 3 respiration in green tomato mitochondria. The LA concentration-dependent decrease in the ADP/O ratio in the presence of BHAM (Table 1) clearly indicates a significant participation of PUMP activity in phosphorylating respiration leading to a strong decrease in the efficiency of oxidative phosphorylation: −18 and −44% at 3.9 and 8 μM LA, respectively.

ADP/O ratios have been used to estimate the contribution of PUMP activity (*i.e.* that part of the cytochrome pathway activity, Vcyt diss, that is finally dissipated into heat) at a given state 3 respiration rate, and to assess the contribution of ATP synthase activity (*i.e.* part of the cytochrome pathway activity, Vcyt cons, that is finally conserved into ATP) at the above two LA concentrations. Thus,

$$\text{Vcyt cons} = \text{V3} \times \frac{(\text{ADP/O})_{+\text{LA}}}{(\text{ADP/O})_{-\text{LA}}} \qquad \text{(Eq. 1)}$$

is the conserving part sustained by ATP synthesis, and

$$\text{Vcyt diss} = \text{V3} - \text{Vcyt cons} \qquad \text{(Eq. 2)}$$

is the dissipating part sustained by PUMP activity. Therefore, if V3 is 500 nmol of oxygen atom/min/mg of protein, Vcyt cons are 412 and 282 nmol of oxygen atom/min/mg of protein, and Vcyt diss are 88 and 218 nmol of oxygen atom/min/mg of protein at 3.9 and 8 μM LA, respectively. These calculations are valid only if several requirements (currently under study) are fulfilled. Nevertheless, the above calculations already show how effective PUMP activity is on energy dissipation and how small variations in the cellular FFA concentration can have a significant influence on oxidative phosphorylation.

A single force-flow relationship has been observed for state 4, state 4_{+olig}, state $4_{+\ LA}$, and state $3_{\pm\ LA}$ (Fig. 3) with succinate (+ rotenone) as oxidizable substrate. This means that modulation of the force (ΔΨ) either by phosphorylation potential or by LA addition leads to the same modification of the flow (respiratory rate). Thus, it can be concluded that the presence of LA gives rise to a proton recycling only, as proposed by Garlid *et al.* (11), and does not interact with the electron transport system in purified tomato fruit mitochondria depleted of endogenous FFA.

In conclusion, we have demonstrated that LA-induced stimulation of respiration is a pure protonophoric effect and that PUMP activity can efficiently divert energy from oxidative phosphorylation in state 3.

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REFERENCES

1. Mitchell, P. (1961) *Nature* **191,** 144–148
2. Vercesi, A. E., Reynafarje, R., and Lehninger, A. L. (1978) *J. Biol. Chem.* **253,** 6379–6385
3. Krämer, R. (1996) *Kidney Int.* **49,** 947–952
4. Lee, C. P., and Ernster, L. (1989) *Biochim. Biophys. Acta* **1000,** 371–380
5. Klingenberg, M. (1990) *Trends Biochem. Sci.* **15,** 108–112
6. Vanlerberghe, G. C., and McIntosh, L. (1997) *Annu. Rev. Plant Physiol. Mol. Biol.* **48,** 703–734
7. Wagner, A. M., and Moore, A. (1997) *Biosci. Rep.* **17,** 319–333
8. Vercesi, A. E., Martins, I. S., Silva, M. A. P., Leite, H. M. F, Cuccovia, I. M., and Chaimovich, H. (1995) *Nature* **375,** 24
9. Vercesi, A. E., Chaimovich, H., and Cuccovia, I. M. (1997) *Recent Res. Dev. Plant Physiol.* **1,** 85–91
10. Skulachev, V. P. (1991) *FEBS Lett.* **294,** 158–162
11. Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, S., and Jezek, P. (1996) *J. Biol. Chem.* **271,** 2615–2620
12. Meese, B. J. D. (1975) *Annu. Rev. Plant Physiol.* **26,** 117–126
13. Day, D. A., Arron, G. P., and Laties, G. G. (1980) in *Biochemistry of Plants* (Davies, D. D., ed) Vol. 2, pp. 197–241, Academic Press, Inc., New York
14. Cruz-Hernández, A., and Gómez-Lim, M. A. (1995) *Planta* **197,** 569–576
15. Van den Bergen, C. W. M., Wagner, A. M., Krab, K., and Moore, A. L. (1994) *Eur. J. Biochem.* **226,** 1071–1078
16. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) *J. Biol. Chem.* **177,** 751–766
17. Kamo, N., Muratsugu, N., Hongoh, R., and Kobatake, Y. (1979) *J. Membr. Biol.* **49,** 105–121
18. Jensen, B. D., Gunter, K. K., and Gunter, T. E. (1986) *Arch. Biochem. Biophys.* **248,** 305–323
19. Ježek, P., Costa, A. D. T., and Vercesi, A. E. (1996) *J. Biol. Chem.* **271,** 32743–32748
20. Ježek, P., Costa, A D. T., and Vercesi A. E. (1997) *J. Biol. Chem.* **272,** 24272–24278
21. Jarmuszkiewicz, W., Sluse-Goffart, C. M., Hryniewiecka, L., Michejda, J., and Sluse, F. E. (1998) *J. Biol. Chem.* **273,** 10174–10180