Malate Metabolism in Leaf Mitochondria from the Crassulacean Acid Metabolism Plant Kalanchoë blossfeldiana Poelln.

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ABSTRACT
The mechanisms and the controlling factors of malate oxidation by mitochondria from leaves of Kalanchoë blossfeldiana Poelln. plants performing Crassulacean acid metabolism were investigated using Percoll-purified mitochondria. The effects of pH and of various cofactors (ATP, NAD*, coenzyme A) on malate dehydrogenase (EC 1.1.1.37) and malic enzyme (EC 1.1.1.39) solubilized from these mitochondria were examined. The crucial role of cofactor concentrations in the mitochondrial matrix on the pathways of malate oxidation is shown. The distribution of the electrons originating from malate between the different electron transport pathways and its consequence on the phosphorylation yield was studied. It was found that, depending on the electron transport pathway used, malate oxidation could yield from 3 to 0 ATP. Assayed under conditions of high reducing power and high energy charge, the ability of malic enzyme to feed electrons to the cyanide-resistant nonphosphorylating alternative pathway was found to be higher than that of other dehydrogenases linked to the functioning of the Krebs cycle (pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase). The physiological significance of such a functional relationship between malic enzyme activity and the nonphosphorylating alternative pathway is discussed in relation to Crassulacean acid metabolism.

Metabolic activity in the leaves of CAM plants is essentially organized around malate (20). Nocturnal activity leads to the accumulation in the vacuoles of large amounts of malic acid that are synthesized through the conjugated actions of PEPC1 and MDH. During the first morning hours, malic acid is released in the cytosol, and then decarboxylated into pyruvate and CO2 through the action of ME. The released CO2 is then made available for the photosynthetic process (20).

In Kalanchoë blossfeldiana leaves, it has recently been shown that mitochondria are implicated in vivo in the malate decarboxylation step (26). Mitochondria can be involved in the process either by reoxidizing the NADP+ generated by the cytosolic NADP+-dependent ME or by reoxidizing the NADH generated by the mitochondrial NAD+-dependent ME (4, 30, 31). In vivo the respective contributions of the two ME to malate decarboxylation remain to be established.

The ability of plant mitochondria to oxidize external (cytosolic) NADPH was initially reported in corn (12). Such a property has been found with many but not all plant species examined, among which are CAM plant mitochondria (2). Available data support the view that this oxidation of NADPH is mediated by a specific NADPH dehydrogenase located on the outer surface of the inner mitochondrial membrane (8). NADPH oxidation has been shown to be sensitive to chelators of divalent cations and to mersalyl, and its regulation has been carefully studied (16).

The involvement of the mitochondrial ME in malate oxidation appears to be more complex (21). Indeed, the knowledge of the general mechanisms of malate oxidation has proved to be of major interest for a better understanding of the functioning of plant mitochondria. After more than 10 years of controversy, a general agreement has been reached concerning the matrix location of MDH and ME (21, 25), the absence of a transhydrogenase putatively involved in the reoxidation of matrix NADH (5), and the fundamental role on the pathways of malate oxidation of the concentrations of cofactors (NAD+, CoA, TPP) in the mitochondrial matrix (5, 13, 15). The problem of a kinetic compartmentation of matrix NAD+ during malate oxidation is still a matter of debate (14, 33).

Previous work had demonstrated the participation of mitochondria in the malate decarboxylation step in vivo in the leaves of K. blossfeldiana (26). The aim of this study on isolated mitochondria was to investigate the mechanisms and the regulation of malate oxidation in vitro, as well as to assess the respective contributions of the dehydrogenases and electron transport pathways involved in this process.

MATERIALS AND METHODS
Plant Material. The experiments were performed on plants (Kalanchoë blossfeldiana Poelln. cv Tom Thumb) grown at the Phytootron in Gif-sur-Yvette (France). The plants were first grown from cuttings under vegetative photoperiodic conditions (16 h light, combined fluorescent and incandescent light; total energy: about 100 W m–2). After 2 months, the plants were transferred to short days (9 h light) for 25 d in order to induce the development of CAM (22). The temperature during the culture was 27°C from 08:45 to 17:45 h and 17°C from 17:45 to 08:45 h. RH was 70%.

Preparation of Mitochondria. Leaf mitochondria were isolated from CAM-performing leaves and further purified on self-generating Percoll gradients as previously described (24). Mitochondria depleted in cofactors (NAD+, CoA, TPP) were obtained by incubating the mitochondria during 15 h at ice-melting temperature as a dilute suspension (about 10 mg protein ml–1) in a medium consisting of 0.3 M mannitol, 1 mM EDTA, and 1 mg ml–1 BSA.

1 Abbreviations: PEPC, phosphoenolpyruvate carboxylase; Ars, arsenite; m-CCCP, carbonyl cyanide m-chlorophenylhydrazone; Isocit, isocitrate; a-KG, a-ketoglutarate; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; PG, propylgallate; RC, respiratory control; SHAM, salicyl hydroxamic acid; state 3 and state 4, oxygen uptake in the presence or after the phosphorylation of ADP; Succ, succinate; TPP, thiamine pyrophosphate.

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Oxygen Uptake and Pyridine Nucleotide Redox State. These parameters were simultaneously measured in a 2.1 ml quartz cell, magnetically stirred, thermostated at 25°C, and placed in a dual wavelength spectrophotometer (Aminco DW 2A). The cell was fitted with a Clark O2 electrode (Beckman 0260 Analyzer). The changes in the pyridine nucleotide redox state were recorded using the wavelength pair 340 and 375 nm. The reaction medium consisted of 0.3 mM mannitol, 10 mM K-phosphate (pH 7.3), 5 mM MgCl2, 10 mM KCl, and 1 g L⁻¹ BSA.

Measurement of Products of Malate Oxidation. The continuous measurement of OAA was carried out using a novel spectrophotometric method, previously described in detail (27). Taking advantage of the high activity of the OAA carrier of the mitochondrial inner membrane (8), this method consists in the study of the equilibrium position of an excess (50 nkat ml⁻¹) of externally added MDH (porcine heart, Sigma) in the presence of external NAD⁺ (400 μM). The medium contained 2 mM EGTA in order to prevent a reoxidation of external NADH by the inner membrane external NADH dehydrogenase and the respiratory chain (16). The enzymic measurement of OAA and pyruvate was carried out according to (25).

Malate Dehydrogenase and Malic Enzyme Activities. Measurement of activities were carried out on detergent-treated mitochondria (Triton X-100, 0.02%, v/v). When measured in the direction of malate formation, MDH (EC 1.1.1.37) activity was assayed by following the oxidation of NADH at 340 nm in a medium consisting of 100 mM phosphate (pH 7.5), 2 mM NADH, and 0.5 mM OAA. In the direction of OAA formation, the activity was measured in the medium used for the O₂ uptake measurements at pH 7.3 in the presence of 100 μM NAD⁺, 10 mM malate, 20 mM glutamate, and an excess (10 nkat ml⁻¹) of aspartate aminotransferase (EC 2.6.1.1) to remove OAA. The ME (EC 1.1.1.39) activity was spectrophotometrically measured by following the reduction of NAD⁺ at 340 nm in the presence of 10 mM phosphate (pH 7.3), 2 mM NAD⁺, and 50 mM malate, unless otherwise indicated. The reaction was started by the successive additions of 5 mM MnCl₂ and 100 μM CoA, obligatory cofactors for this activity (15). Interference with the OAA eventually produced by MDH was avoided by initially adding 25 μM NADH to the reaction medium (11). In order to inhibit the reoxidation of NADH by the respiratory chain 1 mM KCN was present in all assay media for enzymic activities.

Changes in Membrane Potential. Changes in membrane potential were followed spectrophotometrically by the safranine method using the wavelength pair 525 and 558 nm according to (7). Measurements of O₂ uptake were made simultaneously. The experiments were carried out in the medium described for O₂ uptake measurement.

Protein Determination. Protein was determined by mineralization and nesslerization (29).

RESULTS

Properties of Enzymes Involved in Malate Oxidation. The MDH activity of detergent-treated mitochondria (Triton X-100, 0.02%, v/v) amounted to 11,500 nmol min⁻¹ mg⁻¹ protein when measured in the direction of malate formation, and to 1,900 nmol min⁻¹ mg⁻¹ protein when measured in the opposite direction, i.e., malate oxidation. It must be noted that about 15% of this activity was usually measurable prior to the addition of the detergent, whereas generally about 5% of the mitochondria appeared to be damaged, as determined by the permeability of their external membrane toward Cyt c. As previously quoted by Palmer (21), the activity of the MDH, whatever its direction, was considerably higher than the capacity of the respiratory chain to oxidize matrix NADH (about 400 nmol min⁻¹ mg⁻¹ protein). As a consequence the enzyme will rapidly reach its equilibrium position from both directions, and the factors controlling this equilibrium position will be essential for the regulation of malate oxidation. The concentrations of malate and OAA, the redox state of the matrix pyridine nucleotides, and the pH of the medium will greatly affect the equilibrium position of MDH (21). However, as reported for the MDH of mung bean (3, 28) and potato tuber (23) mitochondria, in K. blossfeldiana the activity of MDH was strongly depressed in the presence of ATP: about 65% inhibition of the malate oxidizing activity was observed in the presence of 5 mM ATP and 100 μM NAD⁺. Therefore the equilibrium position will be reached much more slowly when ATP is present in the mitochondrial matrix, i.e., under state 4 conditions.

The ME activity was also strongly dependent on the pH and, when measured in the absence of CoA (Fig. 1A), the highest activity was observed for pH values lower than 7 (15). It is noteworthy that at these low pH values, the measured NADH could originate from the activity of the MDH present in detergent-treated mitochondria rather than from ME activity. Indeed, in the presence of Mn²⁺, a nonenzymic OAA decarboxylation could take place (13) leading to NADH production through the displacement of the equilibrium position of the MDH reaction. However, such a hypothesis can be ruled out since the presence of initially added NADH (see "Materials and Methods") forbids a production of OAA by the MDH present in detergent-treated mitochondria.

The presence of CoA (Fig. 1A) strongly activated the enzyme and shifted the optimum pH to higher values (about 7.3) (6). The maximal activity, measured in the presence of detergent (Triton X-100, 0.02%, v/v), reached 250 to 300 nmol min⁻¹ mg⁻¹ protein. About 5% of the activity of the mitochondria could be measured in the absence of detergent, which corresponded to the percentage of broken mitochondria.

The activation of the enzyme by CoA is illustrated in Figure 1B (at pH 7.3, in the absence of NaHCO₃). Saturation was reached for CoA concentrations higher than 70 μM. As already shown (10, 18), the CoA effect could be balanced by the presence of CoA and NaHCO₃ in the reaction medium.

![Figure 1](image-url)

**Fig. 1.** Factors affecting ME activity solubilized from mitochondria from K. blossfeldiana leaves. A. Effect of 60 μM CoA on ME activity as a function of pH. The activity was measured as described in "Materials and Methods" after solubilization by Triton X-100 (0.02%, v/v). The assay medium included 50 mM malate, 2 mM NAD⁺, 1 mM KCN, and 5 mM MnCl₂. Protein was 0.5 mg ml⁻¹. B. Effect of 3.3 mM bicarbonate (NaHCO₃) on malic enzyme as a function of CoA concentration. Measurement conditions were the same as in A. The pH was 7.3. Protein was 0.3 mg ml⁻¹. C. Inhibition by NADH of malic enzyme activity at pH 7.3. The accumulation of NADH and pyruvate produced by ME was measured under conditions similar to those in B. Samples were withdrawn with time, and their contents in NADH were measured spectrophotometrically, whereas pyruvate was measured in the presence of 0.2 mM NADH and an excess of purified lactate dehydrogenase (10 nkat). Mitochondria (0.27 mg protein) were incubated in 10.1 ml of 10 mM phosphate buffer (pH 7.3).
of NaHCO₃. At the concentration of NaHCO₃ used (3.3 mM), more than 50% inhibition was found for CoA concentrations lower than 50 μM, the inhibitory effect being less with higher CoA concentrations. Finally, the experiment presented in Figure 1C illustrates the inhibitory effect of NADH on ME activity. As measured by the accumulation of NADH and pyruvate, the activity was found to slow down with time. The addition of a limiting amount of OAA (480 μM), sufficient to cause a full reoxidation of the NADH, brought about an important stimulation of the activity of malic enzyme, as judged by the new rate of pyruvate and NADH formation. This set of experiments provides a good overview of the complex interactions between the different factors that will control the activity of malic enzyme in the mitochondria.

Regulation of Mitochondrial Malate Oxidation. The effect of pH on the pattern of malate oxidation is well documented (19, 21) and has been previously studied in *K. blossfeldiana* leaf mitochondria (27). Therefore, the present study was mainly devoted to the problem of the effects of cofactors on malate oxidation. As it is well established that OAA produced by MDH largely controls the pathway of malate oxidation (8), the patterns of O₂ uptake and OAA metabolism were simultaneously studied. In Figure 2A, malate oxidation was studied in the presence of NAD⁺ at pH 7.3. Under these conditions, the mitochondria oxidized malate rapidly as soon as ADP was added and, as shown by OAA determination, the mitochondria did not accumulate OAA. Only after OAA had reached a low level in the external medium, a new addition of ADP led to a slight accumulation of OAA. Then after all ADP was exhausted (state 4), OAA removal took place again.

The effect of NaHCO₃ (3.3 mM) on mitochondrial malate oxidation is shown in Figure 2B. The presence of bicarbonate, which reduces ME activity (Fig. 1B), strongly decreased the oxidation rate and simultaneously induced an accumulation of OAA, by comparison with what was previously observed in the absence of bicarbonate (Fig. 2A). It should be noted that under state 4 conditions the mitochondria retained their ability to eliminate OAA, but under these conditions the residual activity of ME, known to remain active under state 4 conditions (8, 21) (see Table I), produced insufficient amounts of NADH to simultaneously feed electrons to the respiratory chain and to eliminate OAA through its conversion to malate. This was expressed by the biphasic pattern of the O₂ uptake being measured only after most of the previously accumulated OAA had been eliminated. Such a biphasic pattern was not observed in the absence of bicarbonate (Fig. 2A).

In the experiments of Figure 3, carried out with mitochondria depleted of cofactors (see "Materials and Methods"), the respective effects of TPP, arsenite, and CoA were assayed. TPP allowed an activation of pyruvate dehydrogenase and, consequently, increased the rate of removal of OAA through condensation with acetyl CoA, whereas arsenite inhibited this process (Fig. 3A). As previously observed in Figure 2 at pH 7.3 and in the presence of NAD⁺, the OAA level was always very low. Adding arsenite simultaneously caused a progressive decrease in O₂ uptake (about 50%) and an increase in OAA accumulation. Then, the addition of ADP caused a rapid accumulation of OAA that ceased on reaching state 4 conditions. The oxygen consumption was nearly abolished and the OAA concentration then remained stable.

A similar experiment carried out in the presence of CoA instead of TPP is shown in Figure 3B. Under state 3 conditions, OAA accumulation was found to be associated with the oxidation of malate, giving afterward an opportunity to observe the characteristic biphasic kinetics of malate oxidation under state 4 conditions. However, this time, adding arsenite did not affect the pattern of malate oxidation and of OAA metabolism, in contrast with what had been previously observed in the presence of TPP (Fig. 3A). Then, under state 3 conditions, OAA was accumulated less rapidly (comparison of the rates after ADP additions subsequent to arsenite additions in Fig. 3, A and B) and was actively eliminated under state 4 conditions. This pattern of malate oxidation can be easily explained by a stimulation of ME in the presence of CoA (Fig. 1A) which helps remove OAA by generating NADH.

**Malate Oxidation, Electron Transport, and Phosphorylation.** The experiment of Figure 4 shows the phosphorylation yields and changes in membrane potential (as studied by the level of safranine fixation) associated with malate oxidation in relation to state 4.

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**Fig. 2.** Effect of bicarbonate on malate oxidation at pH 7.3 by *K. blossfeldiana* leaf mitochondria. A, *O₂* uptake and changes in oxaloacetate concentrations associated with malate oxidation. Experimental conditions are described in Materials and Methods. B, Effect of 3.3 mM bicarbonate (NaHCO₃) on the same parameters. Experimental conditions were similar in the two experiments. The medium was initially supplemented with MDH (50 nkat), 2 mM EGTA, 400 μM NAD⁺, and 200 μM TPP. Malate was 20 mM, and where indicated 95 μM ADP (final concentration) was added. Numbers along the traces are nmol O₂ min⁻¹ mg⁻¹ protein. Protein was 0.23 mg ml⁻¹.

**Fig. 3.** Effect of TPP, Ars, and CoA on malate oxidation at pH 7.3 by *K. blossfeldiana* leaf mitochondria. A, Antagonistic effects of 200 μM TPP and 3.5 mM Ars on O₂ uptake and changes in OAA concentration associated with malate oxidation. B, Effects of 100 μM CoA and 3.5 mM Ars on the same parameters. Experimental conditions are the same as in Figure 2. Numbers along the traces are nmol O₂ min⁻¹ mg⁻¹ protein. Protein was 0.25 mg ml⁻¹.

**Fig. 4.** Effects of cofactors (ADP, NAD⁺) and inhibitors (cyanide, rotenone, SHAM) on O₂ uptake and membrane potential linked to malate oxidation at pH 7.2. Experimental conditions are described in "Materials and Methods." Membrane potential was measured by the safranine method according to (7). Numbers along the trace are nmol O₂ min⁻¹ mg⁻¹ protein. Protein was 0.23 mg ml⁻¹.
to the electron transfer pathway involved in this oxidation. The addition of the substrate led to a slow O$_2$ uptake and to the establishment of a membrane potential, shown by the fixation of safranine. Adding a limiting amount of ADP caused a decrease in membrane potential concomitant with an increase in O$_2$ consumption. After all ADP had been exhausted membrane potential rose again and O$_2$ uptake was controlled. Similar events were observed following a new ADP addition. The ADP/O ratio values (2.2 to 2.3) indicated that three sites of proton extrusion were involved in the oxidation process.

Then, in the presence of cyanide (under state 4 conditions) a significant decrease in membrane potential was observed, with no change in the rate of O$_2$ uptake. The addition of a limiting amount of ADP led to a new decrease in membrane potential, but did not affect the rate of O$_2$ uptake. No respiratory control was measurable. However, after a short while, membrane potential rose again to a level similar to the one measured before ADP addition. Such changes were repeatedly obtained upon new ADP additions (not shown). Therefore, despite the fact that no RC was measurable, the phosphorylation process was still operating. Under these conditions, malate oxidation was associated with a sufficient proton motive force to allow ATP synthesis, but the activities of MDH and ME were too low for the electron flow to be controlled by the phosphorylation process.

The addition of rotenone led to a new and significant decrease in membrane potential and inhibited the O$_2$ uptake. The subsequent addition of NAD$^+$ increased the O$_2$ uptake, which progressively reached a rate similar to the one measured under state 3 condition in the absence of inhibitors. However, no concomitant increase of membrane potential was observed and ADP addition had no effect on O$_2$ uptake or membrane potential. Under such conditions, the oxidation of malate was not associated to the phosphorylation process (ADP/O = 0). The addition of SHAM, an inhibitor of the cyanide-resistant electron transport pathway, inhibited all O$_2$ uptake but did not decrease the membrane potential. Only the addition of an uncoupler (m-Cl-CCP) collapsed the residual membrane potential.

This experiment establishes that, in the presence of added NAD$^+$, malate oxidation can proceed through a rotenone- and cyanide-resistant electron transfer pathway that bypasses all energy conservation sites. Table 1 shows the respective contributions of MDH and ME to malate oxidation at pH 7.3 (in the presence of Ars), under state 3 and state 4 conditions, and in the presence of cyanide. Under state 3 condition (+ ADP), both enzymes contribute to the oxidation of malate as shown by the accumulation of OAA and pyruvate. Under state 4 condition (+ATP), the production of pyruvate alone accounts for all the O$_2$ consumed. Similarly, in the presence of ATP and cyanide, no OAA is produced by the mitochondria oxidizing malate.

Actually, the high levels of reduction of matrix NADH that are observed in the presence of rotenone (or cyanide) (19), the presence of ATP and the absence of ADP (3), and the absence of an active system for OAA removal will favor a low activity of MDH (27).

The ability of ME to feed electrons to the cyanide-resistant pathway (in the presence of ATP) was compared to the ability of other dehydrogenases linked to the tricarboxylic acid cycle to do so (Fig. 5). After two or three successive additions of ADP and an addition of 500 μM cyanide under state 4 conditions, the rates of oxidation of different substrates were compared to the rate elicited by a subsequent addition of malate. Such an experiment (Fig. 5) carried out with α-ketoglutarate (trace a), pyruvate (in the presence of a sparkier amount of malate) (trace b), or Isocit (trace d) as substrates indicated that the oxidation of these substrates in the presence of cyanide and ATP was rather low compared with the cyanide-resistant O$_2$ uptake induced by malate. Assayed under the same conditions, cyanide-resistant malate oxidation (trace c) was found to be significantly higher (60 nmol min$^{-1}$ mg$^{-1}$ protein compared to 12, 3, and 19 nmol min$^{-1}$ mg$^{-1}$ protein for α-ketoglutarate, pyruvate and Isocit, respectively). Similarly, it was found that, although succinate oxidation was much more resistant to cyanide than exogenous NADH oxidation (trace e), the addition of malate could still increase the O$_2$ uptake taking place in the presence of cyanide. In all cases, PG, another inhibitor of the alternative pathway, fully inhibited the cyanide-resistant oxidation rate.

**DISCUSSION**

The data reported in this study on purified *K. blossfeldiana* leaf mitochondria deal with the two main problems of malate metabolism in plant mitochondria: the interactions between the different enzymes involved in malate oxidation and the interactions of these enzymes with the respiratory chain.

First, they confirm that the metabolism of OAA produced by MDH can be carried out by two processes linked to ME activity. On the one hand, pyruvate produced by ME can be used as a source of acetyl CoA, thus allowing the removal of OAA to yield citrate (Fig. 3A). On the other hand, NADH generated by ME activity can be used for OAA removal through the reversal of the MDH reaction (Fig. 3B). In a previous work carried out with cauliflower bud mitochondria we had come to an opposite conclusion, based on the observation that OAA did not inhibit malate oxidation when ME activity was stimulated by adding NAD$^+$ (which permeates the inner mitochondrial membrane) (5). We now think that the presence of a high concentration of NAD$^+$ (and malate) in the matrix, by stimulating malic enzyme (i.e. high rate of NADH production) and by

![Fig. 5. Resistance to cyanide of the oxidation rates of various substrates by *K. blossfeldiana* leaf mitochondria at pH 7.3. The medium was initially supplemented with 100 μM CoA, 200 μM TTP, 200 μM NAD$^+$, and 2 mM EGTA. Experimental conditions are described in "Materials and Methods." Numbers along the traces are nmol O$_2$ min$^{-1}$ mg$^{-1}$ protein. Protein was 0.6 mg ml$^{-1}$.](image)
decreasing the oxidizing capacity of MDH, would better account both for the resistance of malate oxidation to OAA observed in cauliflower mitochondria in the presence of NAD+ (25) and for the experiment presented here on K. blossfeldiana mitochondria (Fig. 3B).

On the other hand, these results support the view that the involvement of ME in the oxidation of malate could be of crucial importance for the energy balance of the cell. Actually, all the experiments point to the fact that this enzyme, which is tightly controlled by the NAD+ and CoA contents of the mitochondrial matrix, is well suited to carry out the oxidation of malate in the presence of a high energy charge and/or a high reducing power in the mitochondrial matrix (Table I, Fig. 4).

It was also shown that malate dehydrogenase activity is strongly depressed by ATP, as previously observed for the mung bean (3, 28) and potato tuber (23) enzymes. Inasmuch as the cyanide-resistant pathway is considered to operate in vivo only in the presence of a high energy charge in the cell (8), the control of MDH by ATP forbids a significant participation of the mitochondrial MDH in malate oxidation through the cyanide-resistant pathway. Quite similarly, most of the NAD+-dependent dehydrogenases linked to the tricarboxylic acid cycle activity are directly, as α-ketoglutarate dehydrogenase or isocitrate dehydrogenase (32), dependent on the ADP availability in the matrix space. The only other mitochondrial NAD+-dependent enzyme that, like ME, is known to escape such a control is glycine decarboxylase, which is active in K. blossfeldiana mitochondria too (26).

Thus, when assayed under conditions close to those thought to be required for the in vivo functioning of the alternative pathway (i.e. high reducing power and high energy charge), the ability to feed electrons to this pathway appears to be restricted to very few mitochondrial enzymes among which is ME. Such a functional relationship between the activities of ME and alternative pathway has also been observed for the mitochondria from bundle sheaths of C4-plant leaves (9), spadices of Araceae (1) or climacteric fruits (17).

In CAM-plant leaves, the ability of ME to escape control by the energy charge and the reducing power of the cell affords a possibility for the mitochondrial compartment to decarboxylate malate without control by the phosphorylating and reducing activities of the chloroplastic compartment (14, 26).

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