Oxaloacetate and Malate Transport by Plant Mitochondria

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ABSTRACT

The permeability of mitochondria from pea (Pisum sativum L. var Kleine Rheinländerin) leaves, etiolated pea shoots, and potato (Solanum tuberosum) tuber for malate, oxaloacetate, and other dicarboxylates was investigated by measurement of mitochondrial swelling in isosmolar solutions of the above mentioned metabolites. For the sake of comparison, parallel experiments were also performed with rat liver mitochondria. Unlike the mammalian mitochondria, the plant mitochondria showed only little swelling in ammonium malate plus phosphate media but a dramatic increase of swelling on the addition of valinomycin. Similar results were obtained with oxaloacetate, maleate, fumarate, succinate, and malonate. n-Butylmalonate and phenylsuccinate, impermeant inhibitors of malate transport in mammalian mitochondria, had no marked inhibitory effect on valinomycin-dependent malate and oxaloacetate uptake of the plant mitochondria. The swelling of plant mitochondria in malate plus valinomycin was strongly inhibited by oxaloacetate, at a concentration ratio of oxaloacetate/malate of 10^{-3}. From these findings it is concluded: (a) In a malate-oxaloacetate shuttle transferring redox equivalents from the mitochondrial matrix to the cytosol, malate and oxaloacetate are each transported by electrogenic uniport, probably linked to each other for the sake of charge compensation. (b) The transport of malate between the mitochondrial matrix and the cytosol is controlled by the oxaloacetate level in such a way that a redox gradient can be maintained between the NADH/NAD^{+} systems in the matrix and the cytosol. (c) The malate-oxaloacetate shuttle functions mainly in the export of malate from the mitochondria, whereas the import of malate as a respiratory substrate may proceed by the classical malate-phosphate antiport.

Mitochondria from various plant tissues reduce added oxaloacetate at high rates at the expense of NADH generated in the mitochondrial matrix. The reduction of oxaloacetate is so efficient that upon the addition of oxaloacetate to mitochondria oxidizing NADH-linked substrates, such as glycine, respiration can be almost totally inhibited (4, 7, 12, 17, 33). These results led to the proposal that, in these plant mitochondria, a malate-oxaloacetate shuttle may play a physiological role in a transfer of redox equivalents between the mitochondrial matrix and the cytosol. Since the equilibrium of the malate dehydrogenase reaction lies far towards malate formation ($K_{eq} = 3 \times 10^{-5}$ [29]), under physiological conditions the cytosolic malate concentrations are expected to be at least two orders of magnitude higher than the concentrations of oxaloacetate. Therefore, the functioning of a malate-oxaloacetate shuttle under physiological conditions requires that the uptake of oxaloacetate is half-saturated at micromolar concentrations of the substrate and that it is not greatly inhibited by a more than 100-fold excess of malate and other dicarboxylates, which has been demonstrated (14, 21, 34). The ability to catalyze a malate-oxaloacetate shuttle is found in mitochondria from green and nongreen plant tissues. In leaf cells, a malate-oxaloacetate shuttle appears to play a role in providing redox equivalents generated from glycine oxidation in the mitochondria for the reduction of hydroxyacetate located in the peroxisomes (30, 33). In spinach leaves the capacity of malate-oxaloacetate transfer between the mitochondria and the peroxisomes is indeed high enough that at physiological rates of photorespiration all the redox equivalents generated from glycine oxidation could be transferred to the peroxisomes (15).

The mechanisms of the transport of oxaloacetate and malate involved in the malate-oxaloacetate shuttle are not yet clear. Until now, information about the transport systems involved have been obtained by indirect studies only, e.g. from the inhibition of mitochondrial respiration by the addition of oxaloacetate (4, 34), from the concentration dependence of oxaloacetate conversion by intact mitochondria (14, 21), and from the effect of phthalonate, a powerful inhibitor of oxaloacetate uptake, on these parameters (6, 14, 21, 28). It is not known in which way the countertransport of oxaloacetate and malate required for a shuttle is interlinked, e.g. by an antipor catalyzing a counterexchange of the two substrates or by different uniports coupled to each other in some way.

Detailed information about mitochondrial dicarboxylate transport is available from mammalian mitochondria. In mitochondria from rat liver, three different systems for the transport of malate have been well characterized, all functioning as antiport with another anion, namely phosphate, 2-oxoglutarate, and citrate (3, 22–24). In animal mitochondria, oxaloacetate can be transported by the malate transport systems (16, 25), but this transport is very slow and does not appear to play any major role, at least in rat liver and heart (14). It has been reported that in mitochondria from rat kidney a malate-oxaloacetate shuttle may be functioning (26).

In the present report we studied the permeability of plant mitochondria towards oxaloacetate and malate. It will be shown that, in these mitochondria, the major portion of oxaloacetate and malate is not taken up by dicarboxylate-phosphate antiport, as characterized in rat liver mitochondria, but by an electrogenic uniport.

METHODS

Pea seedlings (Pisum sativum L. var Kleine Rheinländerin, obtained from Fa. Van Waveren, Göttingen, FRG) were grown in a greenhouse either in complete darkness or in a 14 h light-10 h dark cycle in moist vermiculite for 12 to 14 d. Potatoes (Solanum tuberosum) were purchased at the local market.

Mitochondria from green pea leaves, etiolated pea shoots, and potato tuber were isolated by the method of Douce et al. (13), as modified by Ebbighausen et al. (14). Rat liver mitochondria were prepared according to Ref. 14. The mitochondria were stored in a medium containing 0.3 M sucrose, 5 mM Tris buffer (pH 7.4), and 1 mM EGTA.

Mitochondrial swelling as an indicator for metabolite uptake

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was measured by the addition of about 50 μl mitochondrial suspension (containing about 0.5–1.2 mg mitochondrial protein) to 1 ml solution containing 5 mM Tes buffer (pH 7.4), 0.1 mM EGTA, 3 μM antimycin A, and 100 mM of a K⁺ or NH₄⁺ salt of the dicarboxylate indicated. When indicated, 5 mM NH₄⁺ phosphate or 10 μM valinomycin were added, temperature 25°C. Immediately after the addition of the mitochondria, the decrease of absorbancy at 546 nm was measured with an Eppendorf photometer.

RESULTS AND DISCUSSION

The Experimental Approach. For a detailed investigation of the properties of malate and oxaloacetate transport in plant mitochondria, we attempted to apply the techniques of silicone layer filtering centrifugation, which had been successfully used earlier by Palmieri et al. (22–24) to characterize the malate-phosphate, malate-citrate, and oxoglutarate-malate translocators of rat liver mitochondria. In these studies, transport was initiated by the addition of radioactively labeled dicarboxylates and terminated by the addition of competitive inhibitors of this transport, immediately followed by separation of the mitochondria from the suspension medium by silicone layer filtering centrifugation. Whereas chloroplasts can be separated from the medium by silicone layer filtering centrifugation within about 2 s, mitochondria, because of their smaller size, may require 30 s for a separation. Therefore, with mitochondria, it is not possible to terminate a reaction as rapid as metabolite transport by silicone layer filtering centrifugation within about 2 s, mitochondria. The validity of the method is totally dependent on the efficiency of the inhibitor stop.

Extensive studies in our laboratory on a possible application of silicone layer filtering centrifugation for measuring dicarboxylate uptake by plant mitochondria led to the unexpected and disappointing result that in these mitochondria phenylsuccinate and butylmalonate did not efficiently inhibit malate uptake. Since we could not obtain a rapid inhibitor stop, we were not in a position to utilize the technique of silicone layer filtering centrifugation for studying the properties of malate and oxaloacetate transport in plant mitochondria, as we had planned. As a substitute we had to make use of the more qualitative swelling techniques for monitoring metabolite uptake. The observation of mitochondrial swelling in isosmotic solutions of dicarboxylate ammonium salts, detected optically as a decrease of light scattering (2), led to the first recognition of a malate-phosphate antiport and an uptake of ammonia via diffusion across the mitochondrial inner membrane. The conversion of NH₄⁺ into NH₃ in the external medium and of NH₃ into NH₄⁺ in the mitochondrial matrix provides the cation for the phosphate taken up and also compensates the efflux of OH⁻ from the mitochondria. From this and other such experiments, it has been established that mitochondria from animal (3) and plant tissues (10, 27, 32) possess a very active phosphate transporter, catalyzed by phosphate/OH⁻ antiport. In mitochondria from pea leaves, an electrogenic uptake of phosphate has also been observed (8).

Rat liver mitochondria do not show any appreciable swelling in isosmotic solutions of ammonium malate unless catalytic quantities of phosphate have been added. For the sake of comparison, we repeated this experiment as shown in Figure 2. Similar results led to the conclusion that the transport of malate is facilitated by malate-phosphate antiport (3). The existence of a malate-phosphate antiport was also established by this swelling technique in mitochondria from plant tissues (27, 32). Further support for the presence of a dicarboxylate-phosphate antiport in plant mitochondria came from uptake measurements by millipore filtration (18) and by back exchange (11).

In our own studies with mitochondria from pea leaves, we also found some stimulation of mitochondrial swelling in ammonium malate by phosphate, but the swelling velocity, related to the swelling in ammonium phosphate alone, appeared to be much lower than with rat liver mitochondria. On the other hand, the swelling in ammonium malate and also in potassium malate was greatly stimulated upon the addition of valinomycin, an ionophore for K⁺ and NH₄⁺ ions (Fig. 3). With K⁺ malate in the presence of valinomycin, the swelling was so rapid that in the experiment of Figure 3 the kinetics are not resolved. A similar experiment in Figure 1 with expanded time scale and depressed absorbancy scale shows that the swelling in K⁺ malate in the presence of valinomycin was even more rapid than the swelling in NH₄⁺ phosphate. In this respect, the plant mitochondria responded differently from rat liver mitochondria, where phos-

![Fig. 1. Pea leaf mitochondria. Swelling in 100 mM K⁺ malate plus 10 μM valinomycin and in 100 mM NH₄⁺ phosphate. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."](image1)

![Fig. 2. Rat liver mitochondria. Swelling in 100 mM NH₄⁺ phosphate, NH₄⁺ malate, and K⁺ malate. When indicated, 5 mM NH₄⁺ phosphate or 10 μM valinomycin were added. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."](image2)
phate is the most rapidly taken up substrate and there is practically no swelling in ammonium or potassium malate in the presence of valinomycin (Fig. 2). These results clearly demonstrate that in the mitochondria from pea leaves the major route of malate uptake is not by malate-phosphate antiport, as in mammalian mitochondria, but by electrogenic uniport, which in the case of our experiment is facilitated by the valinomycin-mediated uptake of the compensating cation. In the pea leaf mitochondria, the activity of the malate uniport appears to be even higher than that of phosphate transport. This malate uniport seems to be a general feature of plant mitochondria. In mitochondria from both etiolated pea shoots (Fig. 4) and potato tuber (Fig. 5), the stimulation of malate swelling by valinomycin was found to be much larger than that caused by phosphate. This was also found when antimycin was omitted from the medium (data not shown). In most cases, however, with mitochondria from etiolated pea shoots or potato tuber, the velocity of valinomycin-dependent swelling in K\(^+\) malate was lower than with mitochondria from green pea leaves. Day and Hanson (5) have published studies with mitochondria from etiolated corn shoots which show that there was a stimulation of malate dependent swelling by valinomycin but did not at the time discuss this observation.

We also studied the swelling of pea leaf mitochondria in isoosmotic K\(^+\) and NH\(_4\)\(^+\) salts of oxaloacetate (Fig. 3). In order to avoid a decomposition of the oxaloacetate in solution, we prepared the solutions a few minutes before the experiments. Although some swelling occurred in NH\(_4\)\(^+\) oxaloacetate alone, the swelling was greatly increased on the addition of valinomycin. There was also a large stimulation of swelling by valinomycin when the mitochondria were suspended in the K\(^+\) salt of oxaloacetate. Compared to this, the stimulation of swelling in NH\(_4\)\(^+\) oxaloacetate by phosphate was small. Similar results were obtained with mitochondria from etiolated pea shoots and from potato tuber (Figs. 4 and 5). Probably due to the lability of

*Fig. 3.* Pea leaf mitochondria. Swelling of mitochondria in NH\(_4\)\(^+\) and K\(^+\) salts of various dicarboxylates (100 mM). When indicated, 5 mM NH\(_4\)\(^+\) phosphate or 10 \(\mu\)M valinomycin were added. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."

*Fig. 4.* Mitochondria from etiolated pea shoots. Swelling of mitochondria in NH\(_4\)\(^+\) and K\(^+\) salts of various dicarboxylates (100 mM). When indicated, 5 mM NH\(_4\)\(^+\) phosphate or 10 \(\mu\)M valinomycin were added. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."
oxaloacetate, data on the swelling of plant mitochondria in oxaloacetate solutions have not been published. Day and Wiskich (6) mentioned an unpublished observation that cauliflower bud mitochondria swell spontaneously when suspended in solutions of NH$_4^+$-oxaloacetate, implying exchange for OH$^-$ ions. Although in our experiments a certain spontaneous swelling in NH$_4^+$ oxaloacetate is observed, the major portion of the oxaloacetate appears to be taken up by electrogenic uniport.

In the experiment of Figure 3, the swelling of pea leaf mitochondria in salts of other dicarboxylates was also measured. A comparison of the results show that there was some stimulation of the swelling in NH$_4^+$ salts by phosphate in the case of succinate and to lesser extent with malonate. Again, there was a considerable increase in swelling in the presence of valinomycin. There was also swelling in salts of fumarate and most rapidly in salts of maleate, which was little affected by the addition of phosphate but greatly stimulated by valinomycin. On the other hand, valinomycin did not stimulate the swelling in K$^+$ salts of 2-oxoglutarate, showing that there is a dicarboxylate specificity in the effect of valinomycin. Similar results were obtained with mitochondria from etiolated pea leaves.

**Effect of Inhibitors.** Whereas n-butylmalonate strongly inhibits phosphate-dependent malate uptake in mitochondria from mammalian (22) and plant (32) tissues, it only slightly affects valinomycin-dependent uptake of malate and oxaloacetate in pea leaves. This is demonstrated in Figure 6. The addition of 10 mM n-butylmalonate resulted in a complete inhibition of the phosphate-dependent swelling of rat liver mitochondria in NH$_4^+$ malate, but there was only a slight effect of the inhibitor on the valinomycin-dependent swelling of pea mitochondria in maleate or oxaloacetate media. The same also applied to phenylsuccinate, another inhibitor of malate-phosphate transport in mammalian mitochondria (22). On the other hand, pea mitochondria swell rapidly in isosmotic concentrations of the K$^+$ salts of both inhibitors, especially in the presence of valinomycin (Fig. 7). This unexpected behavior of the pea mitochondria explains why in our preliminary studies of malate transport with silicone layer filtering centrifugation the inhibitor stop with butylmalonate did not work. We also found a very rapid swelling of pea mitochondria in isosmotic K$^+$ salts of 1,2,3-benzenetricarboxylate, especially when valinomycin was added. 1,2,3-Benzene-tricarboxylate is known as an impermeant inhibitor of the citrate-malate transporter in animal mitochondria (23). The high permeability of this substrate in plant mitochondria may explain the observation of Jung and Laities (18) that 1,2,3-benzenetricarboxylate did not inhibit citrate uptake in mitochondria from potato tuber.

Phthalonate, an inhibitor of oxaloacetate uptake into plant mitochondria (6, 14, 28), had no marked effect on the valinomycin-dependent swelling of pea leaf mitochondria in maleate and oxaloacetate media (Fig. 8). Likewise, Day and Wiskich (6) have mentioned an unpublished observation that the swelling of cauliflower bud mitochondria in NH$_4^+$ oxaloacetate media was not inhibited by phthalonate. The lack of inhibition of swelling in oxaloacetate by phthalonate is probably due to the fact that phthalonate is rapidly taken up by these mitochondria, as indicated by the rapid swelling of pea leaf mitochondria in isosmotic solutions of phthalonate (Fig. 7).

Surprisingly, oxaloacetate turned out to be a strong inhibitor of malate uptake. As shown in Figures 8 and 9, the valinomycin-dependent swelling of pea mitochondria in a solution of 100 mM malate was found to be largely inhibited by 0.1 mM oxaloacetate. No other dicarboxylate, including phthalonate, caused such a strong inhibition. Oxaloacetate also inhibited, though less effectively, the valinomycin-dependent swelling in fumarate salts, but had no effect or only a slight effect on the swelling in other

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**Figure 5.** Mitochondria from potato tuber. Swelling of mitochondria in NH$_4^+$ and K$^+$ salts of malate and oxaloacetate (100 mM). When indicated, 5 mM NH$_4^+$ phosphate or 10 $\mu$M valinomycin were added. The swelling was initiated by addition of mitochondria. For details see "Materials and Methods."

**Figure 6.** Effect of n-butylmalonate and phenylsuccinate on the swelling of mitochondria from etiolated pea shoots in 100 mM K$^+$ maleate plus 10 $\mu$M valinomycin and on the swelling of rat liver mitochondria in 100 mM NH$_4^+$ maleate plus 5 mM NH$_4^+$ phosphate. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."

**Figure 7.** Permeability of mitochondria from etiolated pea shoots to inhibitors. Swelling of mitochondria in K$^+$ salts of phenylsuccinate, n-butylmalonate, and phthalonate (100 mM). When indicated, 10 $\mu$M valinomycin was added. The swelling was initiated by the addition of mitochondria. For details see "Materials and Methods."
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Fig. 8. Effect of oxaloacetate and phthalonate on dicarboxylate uptake. Swelling of mitochondria from etiolated pea shoots in NH₄⁺ salts of malate and oxaloacetate (100 mM) in the presence of 10 μM valinomycin. When indicated, 0.1 mM NH₄⁺ oxaloacetate or 1 mM NH₄⁺ phthalonate were added. The swelling was initiated by the addition of mitochondria. For details see “Materials and Methods.” These inhibition studies were carried out with NH₄⁺ salts for a better resolution of the kinetics.

Fig. 9. Effect of oxaloacetate on dicarboxylate uptake. Swelling of mitochondria from etiolated pea shoots in NH₄⁺ salts of various dicarboxylates (100 mM). When indicated, NH₄⁺ oxaloacetate was added. The swelling was initiated by the addition of mitochondria. For details see “Materials and Methods.” These inhibition studies were carried out in NH₄⁺ salts for a better resolution of the kinetics.

dicarboxylate solutions (Fig. 9). The inhibition of malate transport by oxaloacetate cannot be mimicked by phthalonate (Fig. 8). Earlier measurements of oxaloacetate uptake into plant mitochondria had shown that phthalonate was a strong competitive inhibitor of oxaloacetate uptake. Thus, in mitochondria of pea leaves, the Kᵢ of phthalonate (3 μM) was even lower than the apparent Kᵢ of oxaloacetate for being transported (7 μM) (14).

The similar affinity of the oxaloacetate transport site to phthalonate and oxaloacetate on the one hand, and the lack of interaction of phthalonate instead of oxaloacetate with malate transport on the other hand, suggest that the transport sites for oxaloacetate and malate are different. The inhibitory effect of oxaloacetate on the uptake of fumarate indicates that the malate unipor proposed here also transports fumarate. In this respect, the malate unipor appears to have a specificity different from that of the malate-phosphate antiporter, which does not transport fumarate (22).

Transport Mechanisms Involved in the Malate-Oxaloacetate Shuttle. Our results show striking differences between mitochondrial from rat liver and plant tissues with respect to their dicarboxylate permeability. Thus, plant mitochondria are permeable to inhibitors which cannot penetrate mammalian mitochondrion via the dicarboxylate transporter. This high permeability of plant mitochondria cannot be explained in terms of a membrane damage, since the plant mitochondria used in our studies are of high stability and metabolic competence (see Ebbighausen et al., 14).

Our studies confirm earlier findings that plant mitochondria, like mammalian mitochondria, possess a malate-phosphate antiport. The activity of this transport in the pea mitochondria, however, appears to be relatively low when taking the velocity of swelling as a semiquantitative parameter of uptake rates. This is in contrast to the high capacity of the malate-oxaloacetate shuttle in pea leaf mitochondria (570 nmol/mg mitochondrial protein per min, 25°C) (14). It is therefore most unlikely that in a malate-oxaloacetate shuttle the malate is transported by the above mentioned malate-phosphate translocator.

Both malate and oxaloacetate can penetrate the inner mitochondrial membrane apparently without the requirement of counterexchange with other anions, provided that the anionic charges of the dicarboxylates are compensated, which in our experiments was done by rendering the inner mitochondrial membrane permeable to K⁺ or NH₄⁺ cations by adding valinomycin. Apparently, both malate and oxaloacetate can be transported across the inner mitochondrial membrane by electrogenic unitop. The high velocity of swelling in the presence of valinomycin indicates that, especially in leaf mitochondria, the activity of this transport is very high. This concurs with earlier observations (14) that in mitochondria from leaves the capacity of the malate-oxaloacetate shuttle is higher than in mitochondria from nongreen tissues.

The question of whether both malate and oxaloacetate are transported by a single transport protein, or by two different ones, cannot be decisively answered at present. Earlier studies had shown that butyraldehyde inhibited malate oxidation but had little effect on oxaloacetate reduction by plant mitochondria (6). These findings led to the proposal that oxaloacetate is taken up by a special mechanism apart from the known dicarboxylate transport, but it was not discussed whether the accompanying exflux of malate was catalyzed by the same mechanism (6). As mentioned above, our results indicate that, disregarding whether one or two different transport proteins are involved in a malate-oxaloacetate shuttle, each substrate appears to have at least its special transport binding site. When considering that in a malate-oxaloacetate shuttle both metabolites have to be transported at the same rate although the concentrations of oxaloacetate and malate differ by three orders of magnitude, one might have expected that oxaloacetate and malate bind at different sites.

Summarizing our results, it may be concluded that a malate-oxaloacetate shuttle across the inner mitochondrial membrane is catalyzed by separate electrogenic unipor of malate and oxaloacetate, linked to a counterexchange for the sake of charge compensation (Fig. 10).

Possible Regulation of a Malate-Oxaloacetate Shuttle. A malate-oxaloacetate shuttle has been proposed for the transfer of redox equivalents between the mitochondrial matrix and the cytosol. A highly active shuttle, as present in leaf cells, would result in an equilibration of the redox states of the cytosolic and mitochondrial NADH/NAD systems, unless there were a mechanism to maintain these redox differences. This problem connected with the function of a malate-oxaloacetate shuttle remained unresolved until now and has not even been discussed in the past.

In animal tissues, such as rat liver, the steady-state redox potential of the NADH/NAD in the mitochondrial matrix is about 60 mV more negative than that in the cytosol (1). Whereas the NADH/NAD system in the matrix is about half reduced, it is
almost totally oxidized in the cytosol. The relatively higher redox potential of the NADH/NAD in the matrix appears to be a prerequisite for the respiratory chain function with three coupling sites. In mammalian mitochondria, such as from rat liver, a redox transfer between the mitochondrial and cytosolic NADH/NAD is facilitated by the malate-aspartate shuttle, involving an electrogenic antiport of aspartate with glutamate, which is driven by the membrane potential across the inner mitochondrial membrane. In this way, the proton motive force generated by mitochondrial electron transport maintains the difference in NADH/NAD redox potentials in the two compartments (19).

Recent measurements of subcellular metabolite levels in spinach leaves carried out in our laboratory (B. Riens, D. Heineke, HW Heldt, unpublished data) have indicated that in a leaf cell, in the light and in the dark, the cytosolic NADH/NAD is almost totally oxidized, as in an animal cell. There appears to be an obvious necessity that the very active malate-oxaloacetate shuttle between the leaf mitochondria and the cytosol is prevented from lowering the mitochondrial NADH/NAD redox potential to the low cytosolic one.

This might be achieved by a control of malate transport by oxaloacetate. As shown above, there was a large decrease of valinomycin-dependent swelling of mitochondria in malate media when the concentration ratio of oxaloacetate to malate was 1:1000. This ratio is of physiological significance. In the cytosol of spinach leaves the oxaloacetate/malate ratio has been estimated to be in the range of $10^{-2}$ to $10^{-3}$ (see above). It is feasible that the oxaloacetate present in the mitochondrial matrix may restrict the efflux of malate from the mitochondria in such a way that the ratio of malate/oxaloacetate, and as a consequence also the redox potential of NADH/NAD, could be kept higher than in the cytosol. In this way, the malate-oxaloacetate shuttle could function as a valve, maintaining a difference in redox potentials between the NADH/NAD of the mitochondrial matrix and the cytosol. Such a control of malate transport by oxaloacetate may have the consequence that the uptake of malate from the cytosol into the mitochondria, because of the high cytosolic oxaloacetate level, does not occur by the malate uniport discussed here. The expected redox gradient between the matrix and the cytosol would not allow a redox transfer by the malate-oxaloacetate shuttle from the cytosol into the mitochondria anyway. Therefore, the uptake of malate into the mitochondria as a substrate of mitochondrial oxidation might be expected to occur via the classical malate-phosphate antiport. This would explain why malate-linked respiration is sensitive to butyrylmalonate (6). Although still speculative, these considerations may provide a working hypothesis for an elucidation of the metabolic function of the malate-oxaloacetate shuttle in a plant cell.

These studies, for lack of other suitable methods, had to rely on swelling measurements, employing unphysiologically high substrate concentrations and yielding only semiquantitative information about substrate transport of intact mitochondria. The application of a more quantitative approach for investigating the mechanisms and kinetic properties of malate and oxaloacetate transport in intact mitochondria appeared not possible as there was no inhibitor available for a rapid metabolic stop. It seems now that, even in the case of the availability of a suitable inhibitor, the uptake of dicarboxylates such as malate into leaf mitochondria may be too rapid to be kinetically resolved by even refined techniques of silicone layer filtering centrifugation or milli pore filtration. Therefore, detailed studies on the properties of malate and oxaloacetate transport in leaf mitochondria may require alternative approaches, e.g. measurements of transport after insertion of the mitochondrial transport proteins into liposomes.

LITERATURE CITED