Plant Physiol. (1984) 74, 355–359
0032-0889/84/74/0355/05/$01.00/0

Is the Cytosolic Pi Concentration a Limiting Factor for Plant Cell Respiration?

Received for publication July 26, 1983 and in revised form October 14, 1983

FABRICE REBEILLE, RICHARD BLIGNY, AND ROLAND DOUCE*
Physiologie Cellulaire Végétale. E.R.A. 847, Centre d'Etudes Nucléaires et Université Scientifique et Médicale de Grenoble-85 X, 38041 Grenoble Cédex, France

ABSTRACT

The substrate-dependent O_2 uptake by sycamore (*Acer pseudoplatanus* L.) cell mitochondria in the presence of ADP and limiting Pi concentrations has been measured. The Pi concentration for half-maximum O_2 uptake rate was found to be in the range 20 to 50 micromolar for all the substrates tested. ^{31}P NMR of intact sycamore cells indicated that the Pi concentration in the cytoplasm was in the range 5 to 6 millimolar, approximately 100-fold higher than the Pi concentration required for maximum O_2 uptake rates by isolated mitochondria. When sycamore cells were transferred to a culture medium devoid of Pi, the cytoplasmic Pi concentration decreased from 6 to less than 3 millimolar, but the intact cell respiration remained practically constant for at least 4 days. These results strongly suggest that, *in vivo*, the respiration rate of sycamore cells is not limited by the quantity of Pi supplied to the mitochondria.

Despite much recent progress in elucidating the chemical pathways involved in the respiration of higher plants (7, 8, 26), relatively little information is available concerning the control of rates of O_2 consumption by plant cells. In the cell, the rates of mitochondrial oxidative phosphorylation must be rigorously coordinated to meet the ATP demands of the cytoplasm, and how it is achieved *in vivo* is a cardinal issue in the field of cellular bioenergetics. The rate at which a cell respires can theoretically be limited by any of a number of factors: (a) availability of substrate for the Krebs cycle; (b) availability of Pi or of ADP for oxidative phosphorylation; and (c) the overall capacity of the respiratory chain in a cell.

The flow of substrates across the mitochondrial membrane during respiration is highly controlled and probably none of the metabolically important substrates, except CO_2 and O_2, are able to escape the stringent control exerted by the various specific carriers. In this article, we demonstrate that the mitochondrial phosphate translocator is not ultimately responsible for determining the rate of endogenous respiration in sycamore cells.

MATERIALS AND METHODS

Biological Material. The strain of *Acer pseudoplatanus* L. used in this study was generously provided by J. Guern, plant cellular physiology, Gif sur Yvette, France. The basic nutrient medium was prepared according to Bligny (2) and cell suspensions were maintained in exponential growth by frequent subcultures. The culture medium was kept at a volume of 0.2 L and stirred continuously with a pair of impellers at 60 rpm. The temperature of the experiments was 25°C.

O_2 Consumption. Sycamore cell respiration was measured at 25°C in their culture medium. Unless otherwise mentioned, the reaction medium for mitochondrial respiration contained 0.3 M mannitol; 10 mM KCl, 5 mM $MgCl_2$, 5mM phosphate buffer, pH 7.2, 0.1% defatted BSA. O_2 uptake was monitored polarographically with a Clark-type O_2 electrode system purchased from Hansatech Ltd, King's Lynn, Norfolk, U. K. A value of 240 nmol of O_2/ml was used for the solubility of O_2 at 25°C.

Mitochondrial Preparation. For mitochondrial isolation, mid-log phase cells (500 g) were removed and rinsed three times with 500-ml aliquots of extraction medium containing 0.3 M mannitol. Three rinses were sufficient to remove more than 98% of the extracellular impurities, which hampered mitochondria isolation. Packed cells were then triturated with a sharpened blade Moulinex (3) and were immediately diluted with 2 L of extraction medium. Mitochondria were isolated as described by Bonner (5) and were then purified on discontinuous sucrose gradients (9). The crucial point of the whole preparation is the grinding of the cells. In order to obtain intact mitochondria, it is absolutely necessary to restrict the grinding procedure to a minimum. Rough blending improves the yield of recovered Cyt oxidase or cardiolipin (two mitochondrial specific markers) (3) but increases the proportion of broken mitochondria. The criterion for determining the percentage of intact mitochondria is the comparison of the rate of Cyt *c* oxidation before and after subjecting the preparation to osmotic shock (17). All preparations containing more than 10% damaged mitochondria were discarded. Under these conditions, the final yield of intact mitochondria capable of high state 3 rates of Krebs cycle substrate oxidation (250 nmol O_2/mg protein · min for succinate) is very low (20 mg mitochondrial protein from 500 g packed cells). The final pellet was resuspended at 50 mg of protein/ml, and stored at 2 to 4°C for use within 3 h. Protein concentrations were determined by the method of Lowry *et al.* (15) with BSA as the protein standard.

Silicone Layer-Filtering Centrifugation. The uptake of labeled Pi by intact purified mitochondria was initiated by adding 10 μl of the mitochondrial suspension (about 0.5 mg protein) to 100 μl oxygenated electrode medium containing 2 mM NADH plus a NADH regenerating system (20 mM galactose, 2.5 units/ml galactose dehydrogenase), and either ^{32}Pi (5 mM, 3.7 MBq $mmol^{-1}$), [^{14}C]sucrose (150 MBq $mmol^{-1}$), or ^3H_2O (16.7 kBq ml^{-1}) in a 400-μl capacity polypropylene microtube. The uptake was stopped after 2 min by rapid centrifugation (Beckman, Microfuge B, 10,000*g*) of the mitochondria through a layer of silicone oil (80 μl Versilube F 50, General Electric) into 50 μl 10% $HClO_4$. The top and bottom layers were then separated by cutting the microtubes through the middle of the silicone layer. For details on the filtering centrifugation technique and on the evaluation of the uptake into the sucrose impermeable space (which is the space surrounded by the inner mitochondrial membrane), see Reference 14.

Quantification of Pi. Cell samples (less than 100 mg wet weight)

were harvested rapidly onto a fiber glass filter and washed three times with 10 ml distilled H_2O. For measurement of total Pi, cells were placed immediately into 3 ml of 10% (w/v) TCA and left at 20°C for 10 to 15 min. Each sample was then centrifuged 10 min at 20,000g. Aliquots of the supernatant were removed and Pi was measured by a phosphomolybdic acid method (25).

Phosphorus NMR of Cells. Using a spectrometer (WM 250 Bruker) operating at 101.27 MHz for ^{31}P and the pulsed Fourier transform technique, it is possible to resolve and assign peaks to various intracellular pools of Pi, known to be present in the plant cells (16). By using this method, one may discriminate Pi present in the vacuole (acidic compartment) from that present in the cytoplasm (slightly alkaline compartment). The method, which is noninvasive, relies on the fact that the signals obtained from Pi in different states of ionization occur at different frequencies. Thus, the position of the Pi peaks yields an accurate measure of the degree of protonation of the Pi which in turn yields an accurate measure of the intracellular pH (22). The preparation of cell suspensions to record ^{31}P NMR spectra is fully described elsewhere (16). Cells (1 g wet weight) carefully washed with Pi-free culture medium were introduced in a 15-mm tube with 4 ml of Pi-free buffered culture medium pH 6 to 6.5. The cell suspension was bubbled with air. Spectra were recorded at 101.27 MHz after 1800 accumulations with a repetition time of 1.36 s. The position of the Pi peak was estimated in 5 mM K_2HPO_4/KOH buffers at given pH values. Chemical shifts (ppm) were referenced to orthophosphoric acid. ^{31}P NMR provides not only a nondestructive mean of observing free Pi directly, but also allows an approximation of the relative concentration of Pi present in each major intracellular compartment, *i.e.* the cytoplasm and the vacuole. In several separate experiments, by using KH_2PO_4/KOH buffered solutions at different pH values between 4 and 9, we have observed that there exists a good linear relationship between the Pi peak intensity (area under peaks) and Pi concentration (result not shown). The inaccuracies in determining intracellular Pi concentration are only limited by the uncertainty in determining the area of the wide intracellular Pi resonance peaks.

RESULTS

Figure 1 indicates that sycamore cells harvested after 5 to 7 d of subculture exhibited high rates of O_2 uptake (0.5–0.6 nmol O_2/min·mg wet weight). A respiratory response to FCCP[1] was observed repeatedly at concentrations of 2 μM. There were slight variations in the amount of stimulation observed in different experiments, but the maximum obtained was usually about 100 to 120% above the control. Addition of 100 μM KCN gave rise to a strong inhibition of O_2 consumption. The response to uncouplers indicated that respiration was regulated at some point by the prevailing intracellular concentrations of adenine nucleotides (ADP or ATP) or Pi (24). It is clear, therefore, that sycamore cells are respiring in states between 4 and 3 as defined by Chance and Williams (6). The strong response to KCN indicated that the activity of an alternative, nonphosphorylating, cyanide-insensitive electron transport pathway was negligible in sycamore cells. In order to know if cytoplasmic Pi concentration is the parameter which determines the immediate rates of O_2 consumption, experiments were performed to determine the Pi concentration in the cytoplasmic compartment. Experiments were also undertaken to test whether the phosphate translocator limits the flux to the mitochondria of cytosol Pi and therefore is a rate-limiting step for ATP synthesis in the mitochondria.

Pi Concentration in the Cytoplasmic Compartment. The precise determination of Pi concentration in the cytoplasmic compartment of plant cells is hampered by the presence of an acidic vacuole containing Pi, which occupies much of the cell volume (16). For this purpose, a fruitful approach is to use ^{31}P NMR to discriminate the cytoplasmic from the vacuolar Pi pool (16, 20). The ^{31}P NMR spectrum produced by sycamore cells harvested at the end of the exponential phase of growth (*e.g.* 7-d-old cells) showed two peaks of Pi at 2.1 and 0.33 ppm which are equivalent to values of approximately pH 7.4 and 5.8, respectively (Fig. 2) (16). This reflects the presence of a pool of Pi at acidic pH which is most probably the vacuolar pool and a second pool at a slightly alkaline pH which is cytoplasmic. As the vacuolar peak exhibited an area approximately 2.5-fold higher than that of the cytoplasmic peak, we can therefore conclude, at least for sycamore cells, that the total amount of Pi present in the large vacuolar reservoir is 2.5-fold higher than that of the cytoplasm. Considering the ratio of vacuolar volume to cytoplasmic volume (roughly 9) (4) on the one hand, and the intracellular Pi concentration (approximately 2 mM) on the other hand, we can therefore estimate the cytoplasmic Pi concentration. The value found for the cytoplas-

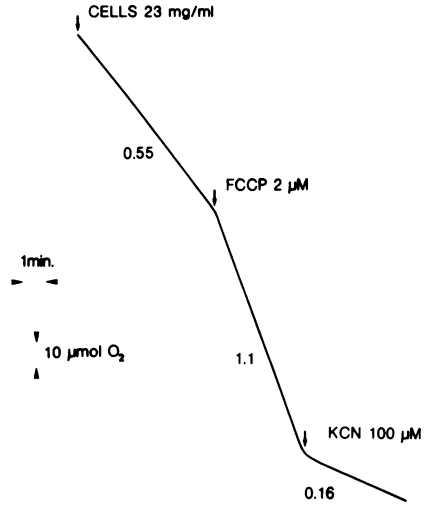

FIG. 1. O_2 consumption by sycamore cells. Cells harvested from the culture medium (23 mg wet weight/ml) were directly introduced in the 1-ml electrode chamber. Numbers along the traces represent the O_2 uptake rates (nmol O_2/min·mg wet weight). Measurement temperature: 25°C. The concentrations given are the final concentrations in the reaction medium.

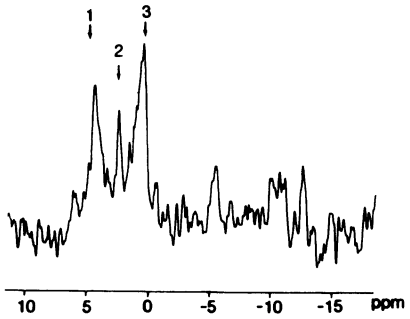

FIG. 2. Representative ^{31}P NMR spectra of sycamore cells harvested at the end of the exponential phase of growth. Cells (about 1 g wet weight) were collected from the culture medium and rinsed three times by successive resuspensions in 25 ml of standard medium buffered at pH 6 to 6.5 by 50 mM MOPS and devoid of Pi. Cells were suspended with 4 ml of the buffered standard medium without Pi directly into the NMR tube. The cell suspension was supplied with O_2 during the experiment. Peaks 1, 2, and 3 were assigned respectively to glucose-6-P and to cytoplasmic (pH 7.5) and vacuolar (pH 5.8) Pi.

[1] Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoro methoxy phenylhydrazone.

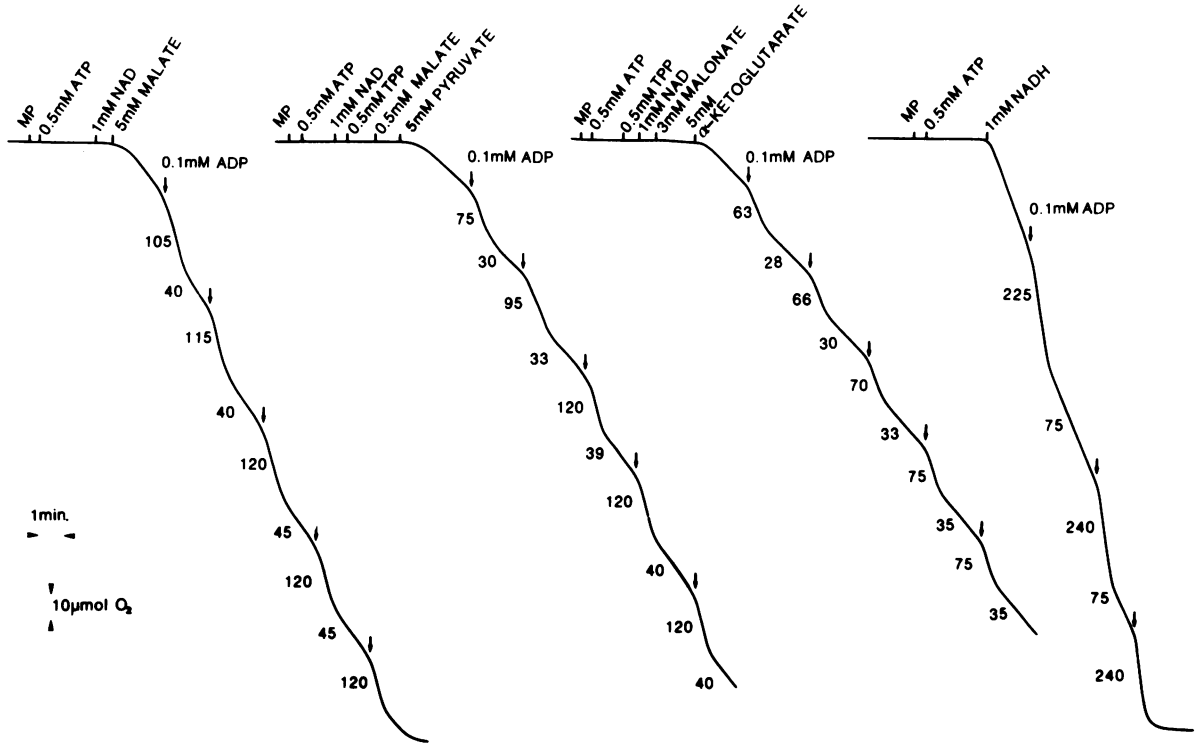

FIG. 3. Oxidation of malate, pyruvate, α-ketoglutarate, and NADH by sycamore cell mitochondria. The concentrations given are the final concentrations in the reaction medium. The numbers on the traces refer to nmol O_2 consumed/min·mg of protein. Measurement temperature: 25°C.

Table I. *Pi Accumulation within Isolated Mitochondria Oxidizing NADH in the Presence of 5 mM Pi*

Conditions for uptake experiments were described under "Materials and Methods." Pi uptake was measured after 4 min either with or without 2 μM FCCP. These values are from a representative experiment and have been reproduced at least three times.

Additions	Pi Concentration within Mitochondria	Pi Concentration in the Medium
	nmol/μl	*mM*
None	14.5	5
FCCP	4.5	5

mic Pi concentration of sycamore cells was in the range 5 to 6 mM.

The question arises whether cytoplasmic Pi might also be segregated within cell organelles such as mitochondria and plastids *in vivo*. In fact, even if Pi accumulates within the mitochondria, on the basis of a proposed Pi ionic charge and pH gradient (21), the cytoplasmic Pi concentration will not be markedly changed, since the small estimated matrix space of mitochondria does not represent more than 5% of the cytoplasmic volume (4). Furthermore, Pi was accumulated only 3-fold within isolated mitochondria oxidizing NADH in the presence of 5 mM Pi (Table I) (see also 7, 11). The uncoupler FCCP caused a sharp decrease in Pi accumulation and reduced the increase of intramitochondrial Pi concentration to a level slightly lower than the concentration in the external medium. Thus, the maximal mitochondrial Pi content would be approximately 0.1 nmol/mg wet weight for a total cytoplasmic Pi concentration of 0.5 nmol/mg wet weight.

In addition, Pi does not seem to accumulate in the plastids since the phosphate translocator situated on the inner membrane of the plastid envelope catalyzes a strict electroneutral counter exchange of Pi^{2-}, 3 P-glycerate^{2-}, glyceraldehyde 3-P^{2-}, and dihydroxyacetone phosphate^{2-} (12).

Table II. *Apparent $K_{m\ Pi}$ of oxidative phosphorylation during oxidation of different substrates in sycamore cells mitochondria.*

The Pi buffer in the electrode medium was replaced by 10 mM MOPS buffer, pH 7.2. Mitochondria were incubated in the presence of 1 mM ADP and 0.5 mM ATP. Malate oxidation was measured in the presence of 1 mM NAD and 5 mM malate; pyruvate oxidation was measured in the presence of 1 mM NAD, 0.5 mM TPP, 0.5 mM malate, and 5 mM pyruvate; α-ketoglutarate oxidation was measured in presence of 1 mM NAD, 0.5 mM TPP, 3 mM malonate, and 5 mM α-ketoglutarate. Pi concentration necessary for half-maximum O_2 uptake rates was measured after adding small amounts of Pi (Pi pulses) to respiring mitochondria (see Fig. 4).

Substrates	$K_{m\ Pi}$
	μM
Pyruvate	20–30
NADH	35–50
α-Ketoglutarate	20–40
Malate	20–40

Control of the Mitochondrial Respiration Rate by the Pi Concentration. Malate, pyruvate, α-ketoglutarate, and NADH were readily oxidized by sycamore cell mitochondria (Fig. 3) with good respiratory controls and ADP/O ratios comparable to those observed with mitochondria from other plant tissues (17). Figure 4 illustrates the response of substrate-dependent O_2 uptake by sycamore cell mitochondria to Pi pulses. These experiments were conducted at 5 mM $MgCl_2$ and 1 mM ADP. It is clear that, with all the substrates used, a Pi concentration of 20 to 50 μM was sufficient for a half-maximal state 3 O_2 uptake rate (Table II). Increasing or decreasing Mg^{2+} concentration by a factor of 5 did not change the apparent $K_{m\ Pi}$. Likewise apparent $K_{m\ Pi}$ values

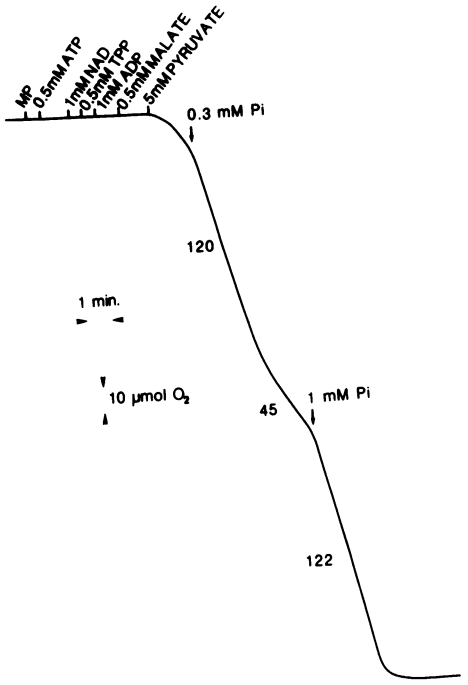

FIG. 4. Oxidation of pyruvate by sycamore cell mitochondria in the presence of Pi-limiting concentrations. The concentrations given are the final concentrations in the reaction medium. The numbers on the traces refer to nmol O_2 consumed/min·mg of protein. Measurement temperature: 25°C. In these conditions, Pi buffer in the electrode medium was changed by 10 mM MOPS, pH 7.2.

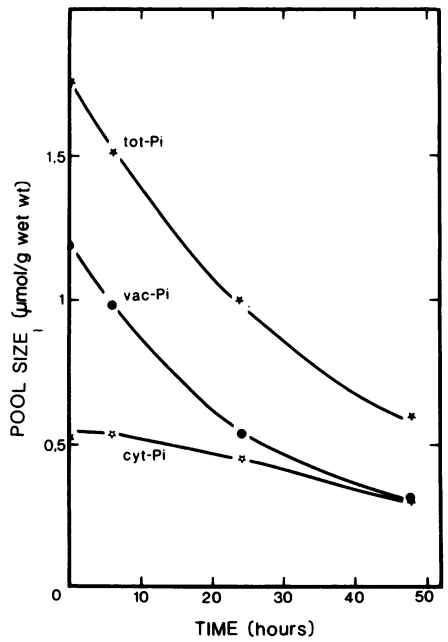

FIG. 5. Effect of Pi starvation on the vacuolar and cytoplasmic Pi content of sycamore cells. Cells were harvested from the culture medium and rinsed three times by successive resuspensions in fresh culture medium devoid of Pi. Cells were incubated at time 0 into flasks containing Pi-free culture medium. At various times, total Pi was measured by a phosphomolybdic acid method and vacuolar and cytoplasmic Pi contents were calculated from NMR spectra (see "Materials and Methods"). tot, vac, and cyt are total, vacuolar, and cytoplasmic, respectively.

Table III. *Time Course of Sycamore Cell Respiration Rates When Cells Are Grown in a Medium Devoid of Pi*

Cells were harvested from the culture medium and rinsed three times by successive resuspensions in fresh culture medium devoid of Pi, then incubated in flasks containing Pi-free culture medium. The slight variation (15%) of respiratory rates between time 0 and 72 h is not significant since during the same time, the cell volume may increase slowly as the growth rate decreases (2). Uncoupled respiration rates were obtained with 2 µM FCCP.

Time	Respiratory Rates	Uncoupled Respiration Rates
h	*nmol O_2/min·mg wet wt*	
0	0.5	0.95
24	0.5	1
48	0.47	1
72	0.42	0.85

are somewhat independent of ADP and ATP concentrations (100 µM to 4 mM) in the medium (result not shown). The apparent $K_{m\ Pi}$ for respiratory stimulation is approximately 2 orders of magnitude lower than the value of cytoplasmic Pi concentration (5–6 mM). It may be assumed, therefore, that the cytosolic Pi concentration is in large excess and should exert no major control on the *in vivo* rates of respiration or oxidative phosphorylation.

Effect of Pi Starvation on Sycamore Cells. To test further whether the cytosolic Pi concentration is rate-limiting for oxidative phosphorylation in sycamore cells, experiments were undertaken to decrease the cytoplasmic Pi pool. When Pi was omitted from the sycamore cell culture medium, the endogenous Pi decreased to 10% of the control within 72 h (18). Under these conditions, ^{31}P NMR spectra were obtained from intact sycamore cells to determine the Pi concentration in each intracellular compartment (*i.e.* the vacuole and the cytosol). During the first hours of Pi starvation, the vacuolar Pi pool considerably decreased relative to the cytoplasmic Pi pool (Fig. 5) (19). This result suggests that during the first hours of Pi starvation, the Pi content of the cytoplasm is largely maintained at the expense of the vacuolar pool. Once the vacuole has been emptied of its Pi content, the cytoplasmic Pi pool decreased more steadily. Consequently, the omission of Pi from the nutrient solution lowered the cytoplasmic Pi concentration from 5 to 6 to 3 mM in 48 h. The ^{31}P NMR technique is not sensitive enough to determine the Pi in the cytoplasm and in the vacuole after 48 h, but total cell Pi concentration, determined by a phosphomolybdic acid method, decreased continuously down to 0.3 mM after 3 d of Pi starvation (18). Cell growth stopped completely when a threshold of cytoplasmic Pi concentration was attained. Under these circumstances, it is possible that not all of the cytoplasmic Pi pool can be used for growth because it is an obligatory anion necessary to sustain various metabolic sequences. If we assume that cytoplasmic Pi exerts a tight control on the rate of respiration, it might be anticipated that decreased cytoplasmic Pi would lead to a decreased respiration rate. In fact, Table III shows that the rate of O_2 consumption by sycamore cells maintained in a medium devoid of Pi did not decrease significantly with time. Likewise, the uncoupled rate of O_2 consumption was not affected as the cytoplasmic Pi concentration decreased. Together, these results suggest that cytoplasmic Pi concentration does not limit, *in vivo*, oxidative phosphorylation.

DISCUSSION

The data reported in this paper indicate that, in physiological situations, the respiration rate of *Acer pseudoplatanus* cells is not limited by the quantity of Pi supplied to the mitochondria. The

two major arguments in favor of this conclusion are:(a) the external Pi concentration necessary for a maximal O_2 uptake rate by isolated sycamore cell mitochondria is approximately 100-fold lower than the value of 5 to 6 mM for cytoplasmic Pi concentration found in sycamore cells. (b) Decreasing cytoplasmic Pi concentration does not affect the rate of O_2 consumption by intact cells.

One must distinguish, when considering rate control by carriers, between rate limitation by the capacity of the carrier on the one hand and the availability of the substrate on the other hand (23). Thus, it is possible that in the case of Pi translocation, the capacity of the carrier can limit the rate of oxidative phosphorylation. However, this hypothesis is unlikely because the capacity of the Pi carrier, as measured by direct transport of labeled Pi in isolated mitochondria (7), is much higher than the maximum measured rate of oxidative phosphorylation. Furthermore, as the K_m value of Pi for the translocator is between 200 and 300 μM (11) and the cytoplasmic Pi content is between 5 and 6 mM, the translocator could operate at full capacity and thus, in physiological situations, the capacity well exceeds the requirement. Thus, in the case of the Pi translocator, neither the capacity of the carrier nor the rate of supply of Pi to the electron transport chain, limits the rate of respiration. In fact, the results presented by Jacobus *et al.* (13) for mammalian mitochondria and Dry and Wiskich (10) for plant mitochondria, strongly suggest that the most plausible explanation of respiratory control is the availability of ADP and the kinetics of its transport by the adenine nucleotide translocase, a hypothesis first proposed by Chance and Williams (6).

Acknowledgments—We thank Professor J. Guern and Dr. J. B. Martin for their keen interest and encouragement.

LITERATURE CITED

1. AP REES T 1980 Assessment of the contributions of metabolic pathways to plant respiration. *In* DD Davies, ed, The Biochemistry of Plants. Academic Press, New York, Vol 2, pp 1–29
2. BLIGNY R 1977 Growth of suspension cultured *Acer pseudoplatanus* L. cells in automatic culture units of large volume. Plant Physiol 59: 502–505
3. BLIGNY R, R DOUCE 1980 A precise localization of cardiolipin in plant cells. Biochim Biophys Acta 617: 254–263
4. BLIGNY R, R DOUCE 1976 Les mitochondries de cellules végétales isolées (*Acer pseudoplatanus* L.). Physiol Veg 14: 499–515
5. BONNER WD 1967 A general method for the preparation of plant mitochondria. Methods Enzymol 10: 126–133
6. CHANCE B, GR WILLIAMS 1955 Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen evolution. J Biol Chem 217: 385–393
7. COTY WA, PL PEDERSEN 1974 Phosphate transport in rat liver mitochondria. J Biol Chem 249: 2593–2598
8. DAY DA, GP ARRON, GG LATIES 1980 Nature and control of respiratory pathway in plants. The interaction of cyanide-resistant respiration with the cyanide-sensitive pathway. *In* DD Davies, ed, The Biochemistry of Plants. Academic Press, New York, Vol 2, pp 198–241
9. DOUCE R, EL CHRISTENSEN, WD BONNER 1972 Preparation of intact plant mitochondria. Biochim Biophys Acta 275: 148–160
10. DRY IB, JT WISKICH 1982 Role of the external adenosine triphosphate/adenosine diphosphate ratio in the control of plant mitochondrial respiration. Arch Biochem Biophys 217: 72–79
11. HANSON JB, BL BERTAGNOLLI, WD SHEPHERD 1972 Phosphate-induced stimulation of acceptorless respiration in corn mitochondria. Plant Physiol 50: 347–354
12. HELDT HW 1976 Transfer of substrates across the chloroplast envelope. Horiz Biochem Biophys 2: 199–229
13. JACOBUS WE, RW MOREADITH, KM VANDEGAER 1982 Mitochondrial respiratory control. J Biol Chem 257: 2397–2402
14. KLINGENBERG M, E PFAFF 1967 Means of terminating reactions. Methods Enzymol 10: 680–684
15. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
16. MARTIN JB, R BLIGNY, F REBEILLE, R DOUCE, JJ LEGUAY, Y MATHIEU, J GUERN 1982 A ^{31}P nuclear magnetic resonance study of intracellular pH of plant cells cultivated in liquid medium. Plant Physiol 70: 1156–1161
17. NEUBURGER M, EP JOURNET, R BLIGNY, JP CARDE, R DOUCE 1982 Purification of plant mitochondria by isopycnic centrifugation in density gradients of Percoll. Arch Biochem Biophys 217: 312–323
18. REBEILLE F, R BLIGNY, R DOUCE 1982 Regulation of Pi uptake by *Acer pseudoplatanus* cells. Arch Biochem Biophys 219: 371–378
19. REBEILLE F, R BLIGNY, R DOUCE 1983 Relationship between the cytoplasm and the vacuole phosphate pool in *Acer pseudoplatanus* cells. Arch Biochem Biophys 225: 143–148
20. ROBERTS JKM, PM RAY, N WADE-JARDETZKY, O JARDETZKY 1980 Estimation of cytoplasmic and vacuolar pH in higher plant cells by ^{31}P NMR. Nature (Lond) 283: 870–872
21. SCARPA A 1979 Transport across mitochondrial membranes. *In* G Giebisch, DC Tosteson, HH Ussing, eds, Membrane Transport in Biology. Springer-Verlag, Berlin, Vol II, pp 263–355
22. SCHULMAN RG 1983 NMR spectroscopy of living cells. Sci Am 248: 76–83
23. STUBBS M, PV VIGNAIS, HA KREBS 1978 Is the adenine nucleotide translocator rate-limiting for oxidative phosphorylation? Biochem J 172: 333–342
24. TAGER JM, RJA WANDERS, AK GROEN, W KUNZ, R BOHNENSACK, U KUSTER, G LETKO, G BÖHME, J DUSZINSKI, L WOJTCZAK 1983 Control of mitochondrial respiration. FEBS Lett 151: 1–9
25. Taussky H, E Shorr 1953 A microcolorimetric method for the determination of inorganic phosphorus. J Biol Chem 202: 675–685
26. TURNER JF, DH TURNER 1980 The regulation of glycolysis and the pentose phosphate pathway. *In* DD Davies, ed, The Biochemistry of Plants. Academic Press, New York, Vol 2, pp 279–316