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TRANSPORT OF PYRUVATE AND LACTATE IN YEAST MITOCHONDRIA

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SUMMARY

Evidence for the existence of mediated transport of pyruvate and lactate in isolated mitochondria of *Saccharomyces cerevisiae* is presented.

1. The mitochondrial oxidation of pyruvate is specifically inhibited by the monocarboxylic oxoacids α-ketovalerate, α-ketoisocaproate and by α-cyano-3-hydroxycinnamate, while pyruvate and malate dehydrogenases activities are not inhibited.

2. The stimulation of the mitochondrial oxidations of succinate, α-ketoglutarate and citrate by pyruvate are also inhibited by α-cyano-3-hydroxycinnamate.

3. The [^{14}C]pyruvate uptake by yeast mitochondria follows saturation kinetics and is completely inhibited by α-cyano-3-hydroxycinnamate.

4. Large amplitude passive swellings of mitochondria of the wild type and of cytoplasmic rho$^-$ and rho$^-$ n mutants are induced by isoosmotic ammonium pyruvate and lactate. These pH-dependent swellings are inhibited by α-cyano-3-hydroxycinnamate suggesting that the carrier system is not coded by mitochondrial DNA.

INTRODUCTION

The mitochondrial transport of pyruvate by a specific monocarboxylate carrier as proposed by Papa et al. [1] has been questioned on the basis that sufficient undissociated lipid-soluble monocarboxylic acids could diffuse freely across the mitochondrial membrane [2, 3]. However, recent studies by Papa and Paradies [4, 5] and Halestrap et al. [6] have confirmed the existence of a specific mediated transport of pyruvate in rat liver mitochondria and in the plasma membrane of human erythrocytes by showing that this transport was specifically inhibited by α-cyano-4-hydroxycinnamate. It has been suggested that the carrier involved in the transport of pyruvate might also be implied in lactate transport in erythrocytes, though not in rat liver mitochondria [6]. Further support to the existence of a lactate translocator has been given in a recent paper by Spencer and Lehninger who reported the inhibition of lactate transport in ascites tumour cells by α-cyano-4-hydroxycinnamate or substituted monocarboxylic acids [7]. The presence of specific carriers for pyruvate and for

lactate in the mitochondrial membrane of yeasts has not been reported so far. The major reason to investigate the presence in yeast of these translocators, which must play a central role in the regulation of cell catabolism, is that yeasts can easily be submitted to elaborate physiological and/or genetic manipulations which can modify the composition of the mitochondrial membrane.

This paper reports evidence for the presence of a pyruvate translocator in the mitochondria of wild type and in respiratory-deficient cytoplasmic petite (rho^-) and neutral petite (rho^-n) mutants of *Saccharomyces cerevisiae*. Evidence indicating the existence of a mediated transport for lactate in these mitochondria is also provided.

MATERIALS AND METHODS

Mitochondria were isolated as previously described [8] from three strains of the yeast *Saccharomyces cerevisiae*; the diploid wild type strain D261, the suppressive respiratory-deficient cytoplasmic petite IL166-6C (*a ura*1 rho^-) and the neutral cytoplasmic petite IL166-6C (*a ura*1 rho^-n) obtained by ethidium bromide treatment of the IL166-6C (*a ura*1 rho^+) haploid strain. Cells were grown aerobically for 18 h in a fermentor at 30 °C in medium containing 1 % yeast extract (Difco), 0.2 % ammonium phosphate, 0.2 % ammonium sulfate, 0.1 % glucose, supplemented with 3 % ethanol for the strain D261 or by 5 % glucose for the two cytoplasmic petite mutants. The mitochondria of the petite mutants lack detectable cytochromes *b*, $a+a_3$ and oligomycin-sensitive ATPase.

Mitochondrial respiration was measured at 25 °C with a Clark oxygen electrode in a 3-ml closed cell containing 0.6 M mannitol, 10 mM potassium phosphate, 10 mM imidazole-HCl (pH 6.4) and 0.1 % bovine serum albumin. Pyruvate dehydrogenase (EC 1.2.4.1) and malate dehydrogenase (EC 1.1.1.37) activities were measured in Triton X-100 treated mitochondria as described previously [8].

Pyruvate uptake by isolated mitochondria incubated with [^{14}C]-pyruvate was measured by liquid scintillation counting in $HClO_4$ extract of the pellet and the supernatant obtained by rapid centrifugation of the incubation medium (see legend of Fig. 2). The pyruvate content of the mitochondrial matrix was corrected as described by Papa et al. [9] using [^{14}C]-sucrose in order to account for the extramitochondrial pyruvate enclosed in the pellet.

Swelling of mitochondria was carried out at 25 °C in 0.2 M ammonium pyruvate and L(+)-lactate in the presence of 5 μg antimycin A and 10 mM 2-(*N*-morpholino)ethane sulfonic acid buffer at pH 6.5. Protein was measured by the method of Waddel [10].

RESULTS AND DISCUSSION

Effect of monocarboxylic oxoacid analogues and of α-cyano-3-hydroxycinnamate on the oxidation of pyruvate by yeast mitochondria .

The possibility of a mediated transport of pyruvate was suspected after the observation (Table I) that the state 3 oxidation rate of pyruvate by yeast mitochondria of the strain D261 is specifically inhibited by 5 mM of monocarboxylic oxoacids such as : α-ketovalerate, α-ketoisocaproate and by 50 μM of α-cyano-3-hydroxycinnamate.

TABLE I

EFFECT OF MONOCARBOXYLIC OXOACIDS AND OF α-CYANO-3-HYDROXYCINNAMATE ON THE STATE 3 RATE OF YEAST MITOCHONDRIAL OXIDATIONS

Mitochondria (1.2 mg protein) were preincubated for 2 min at 25 °C in the mannitol reaction mixture described in Materials and Methods in the presence or absence of 5 mM of the oxoacids and 50 μM of α-cyano-3-hydroxycinnamate. 5 mM of the respiratory substrate (plus 0.5 mM malate with pyruvate as substrate) were then added and after 1 min further incubation, the state 3 was induced by the addition of 0.2 mM ADP.

Addition	Pyruvate oxidation*	Inhibition (%)	Succinate oxidation*	Inhibition (%)	α-Ketoglutarate oxidation*	Inhibition (%)	Isocitrate oxidation*	Inhibition (%)	D(−)-Lactate oxidation*	Inhibition (%)	L(+)-Lactate oxidation*	Inhibition (%)
None	195	–	206	–	120	–	62	–	38	–	18	–
α-Cyano-3-hydroxycinnamate	59	70	206	0	121	0	62	0	38	0	17	7
α-Ketocaproate	51	74	208	0	43	68	54	13	17	55	13	30
Phenylpyruvate	97	50	162	21	52	40	46	25	29	23	10	44
α-Ketovalerate	92	53	205	0	107	11	61	1	39	0	17	5
α-Ketobutyrate	162	17	233	0	111	7	62	0	26	31	80	0
α-Ketoisocaproate	131	33	203	1	114	5	62	0	43	0	19	0
α-Ketoisovalerate	132	32	213	0	68	43	54	13	28	26	17	5

* The rate of oxidation is expressed as natoms oxygen per min per mg.

TABLE II

EFFECT OF MONOCARBOXYLIC OXOACIDS AND OF α-CYANO-3-HYDROXYCINNAMATE ON THE PYRUVATE AND MALATE DEHYDROGENASE ACTIVITIES OF YEAST MITOCHONDRIA

Mitochondria (0.8 mg protein) were preincubated for 2 min at 25 °C in the reaction mixture as indicated under Materials and Methods in the presence of 5 mM of the oxoacids or 50 μM of α-cyano-3-hydroxycinnamate, or 250 μM mersalyl or *N*-ethyl-maleimide. The reaction was then initiated by addition of pyruvate or oxalacetate.

Addition	Pyruvate dehydrogenase (nmol $NAD^+ \cdot min^{-1} \cdot mg^{-1}$)	Inhibition (%)	Malate dehydrogenase (μmol $NADH \cdot min^{-1} \cdot mg^{-1}$)	Inhibition (%)
None	156	–	5.0	–
α-Cyano-3-hydroxycinnamate	156	0	5.1	0
α-Ketocaproate	137	12	5.0	0
Phenylpyruvate	132	15	5.0	0
α-Ketovalerate	140	10	5.1	0
α-Ketoisocaproate	156	0	5.0	0
α-Ketoisovalerate	101	35	5.4	0
Mersalyl	30	81	4.8	4
N-Ethyl-maleimide	108	31	5.2	0

TABLE III

COMPARATIVE EFFECT OF THIOL REAGENTS AND OF α-CYANO-3-HYDROXYCINNAMATE ON THE STATE-4 RATE OF YEAST MITOCHONDRIAL OXIDATIONS

Mitochondria (1.2 mg protein) were preincubated for 2 min at 25 °C in the mannitol reaction mixture described in Materials and Methods in the presence or absence of 250 μM mersalyl or 250 μM N-ethyl-maleimide, or 100 μM α-cyano-3-hydroxycinnamate. The respiratory substrate (5 mM) was then added to initiate the state 4 of oxidation.

Addition	Pyruvate oxidation*	Inhibition (%)	Succinate oxidation*	Inhibition (%)	Isocitrate oxidation*	Inhibition (%)	D(−)-Lactate oxidation*	Inhibition (%)	L(+)-Lactate oxidation*	Inhibition %
None	40	–	134	–	69	–	30	–	19	–
N-Ethyl-maleimide	23	42	59	56	44	35	23	24	15	21
Mersalyl	30	25	55	59	15	77	17	43	8.6	55
α-Cyano-3-hydroxy-cinnamate	10	75	134	0	69	0	31	0	15	21

* Oxidation rates are expressed in natom oxygen per min per mg.

TABLE IV

INHIBITION BY α-CYANO-3-HYDROXYCINNAMATE OF THE MITOCHONDRIAL OXIDATIONS STIMULATED BY PYRUVATE

Mitochondria (0.7 to 1.4 mg protein) were incubated with or without 100 μM α-cyano-3-hydroxycinnamate for 2 min at 25 °C in the mannitol reaction mixture given under Materials and Methods. 16.5 mM succinate or α-ketoglutarate or citrate were then added and after 1 min further incubation, state 3 was initiated by addition of 0.2 mM ADP. When used, 1 mM pyruvate was added 30 s before addition of ADP.

Addition	State 3 oxidation rates (natom oxygen · min^{-1} · mg^{-1})		
	In absence of pyruvate	In presence of pyruvate	Stimulation by pyruvate
Succinate	200	309	109
Succinate + α-cyano-3-hydroxycinnamate	191	216	25
α-Ketoglutarate	165	230	65
α-Ketoglutarate + α-cyano-3-hydroxycinnamate	165	176	11
Citrate	108	172	64
Citrate + α-cyano-3-hydroxycinnamate	108	120	12

These compounds have been previously described as specific inhibitors of pyruvate transport in rat liver mitochondria and in human erythrocytes [5, 6, 11].

Other oxoacids also inhibit the oxidation of pyruvate but are less specific since the oxidations of succinate, α-ketoglutarate, isocitrate or D(-) and L(+) lactate are also affected to variable extents. The specific inhibition of pyruvate oxidation by α-ketovalerate, α-ketoisocaproate and α-cyano-3-hydroxycinnamate is interpreted as an inhibition of pyruvate transport since pyruvate and malate dehydrogenases are not significantly inhibited by these compounds as shown in Table II.

It has been reported by Papa et al. [4] that pyruvate translocation in rat liver mitochondria is sensitive to the thiol blocking reagents mersalyl and *N*-ethyl-maleimide. Table III shows that the state 4 oxidation rates of pyruvate, as well as of succinate, isocitrate or D(-) and L(+)-lactate by yeast mitochondria are sensitive to these thiol reagents. Moreover, both these reagents inhibit the mitochondrial pyruvate dehydrogenase activity (Table II). Therefore they cannot be used as specific inhibitors of the pyruvate transport.

It has been shown that pyruvate stimulates the oxidation of succinate in rat liver mitochondria [12]. This stimulation is probably due to the elimination of the intramitochondrial oxalacetate [13, 14]. Table IV illustrates that in yeast mitochondria, pyruvate stimulates markedly the state 3 oxidation rates not only of succinate but also of α-ketoglutarate and citrate. This table shows that the extra oxygen uptake observed in the presence of pyruvate is about 80 % inhibited by 0.1 mM α-cyano-3-hydroxycinnamate, again confirming a possible specific action of this inhibitor on pyruvate transport.

Pyruvate transport

As shown in Fig. 1, passive swelling of yeast mitochondria is observed in

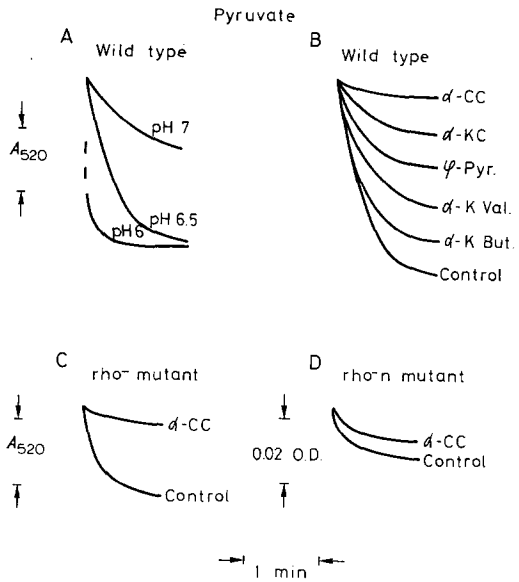

Fig. 1. Swelling of mitochondria in ammonium salt of pyruvate. The mitochondria (1.5 mg protein) were preincubated in the mannitol reaction mixture for 2 min at 25 °C with or without the inhibitor under study. An aliquot of the mixture (0.5 mg protein) was then added to 1 ml reaction mixture containing 0.2 M ammonium pyruvate, 10 mM 2-(*N*-morpholino) ethanesulfonic acid and 5 μg of antimycin A. The swelling was monitored by measuring the decrease in absorbance (*A*) at 520 nm at 25 °C and at pH 6.5 when not specified in the figure. (A) Effect of pH on the swelling of fully functional yeast mitochondria (strain D261). (B) Effect of 20 mM α-ketobutyrate (α-K But), α-ketovalerate (α-K Val), phenylpyruvate (ψ-Pyr), α-ketocaproate (α-KC) and of α-cyano-3-hydroxycinnamate (α-CC) on the swelling of fully functional mitochondria. (C) and (D), effect of 20 mM α-cyano-3-hydroxycinnamate on the swelling of mitochondria of the respiratory deficient mutants rho$^-$ and rho$^-$n.

isoosmotic ammonium pyruvate. In agreement with the results obtained with rat liver mitochondria by Brouwer et al. [15], we observe (Fig. 1A) that the rate of swelling of yeast mitochondria is increased by lowering the pH from 7.0 to 6.0, indicating that the uptake of pyruvate is coupled to proton influx or hydroxyl efflux. This swelling is inhibited by preincubation of mitochondria for 2 min in the presence of 20 mM of oxoacids. Among them, the efficiency of inhibition of the pyruvate-induced swelling paralleled that of the state 3 oxidation rates of pyruvate, with 20 mM α-cyano-3-hydroxycinnamate exhibiting the most prominent effect (Fig. 1B and Table I).

Under similar conditions, α-cyano-3-hydroxycinnamate, which inhibits 90 % of the pyruvate-induced swelling, inhibits by only 20 % the succinate (plus phosphate) swelling, by 12 % the citrate (plus malate and phosphate) swelling, by 40 % the malate (plus phosphate) swelling and by 50 % the oxoglutarate (plus malate and phosphate) swelling. The significant inhibition of the transport of malate and oxoglutarate might be due to inhibition of the phosphate carrier, since swelling in ammonium phosphate is 45–50 % inhibited by 20 mM α-cyano-3-hydroxycinnamate (data not shown).

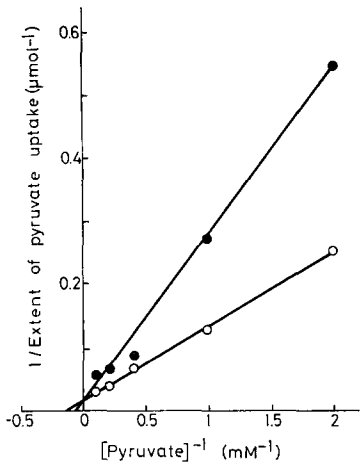

Fig. 2. Double reciprocal plot of the effect of 1 mM α-cyano-3-hydroxycinnamate on the total [^{14}C]-pyruvate uptake in yeast mitochondria. Mitochondria (6.8 mg protein) from the wild type strain D261, were preincubated for 2 min at 10 °C in the medium containing 0.6 M sorbitol, 0.5 mM EDTA, 0.1 % bovine serum albumin, 2.5 μg/ml antimycin A, 12.5 μg/ml oligomycin and 10 mM piperazine-*N*,*N*′-bis-(2-ethane sulfonic acid), pH 6.4. [^{14}C]pyruvate was then added and after 1 min incubation, the mitochondria were separated from the reaction mixture by rapid centrifugation at 40000 × *g* for 2 min at 2 °C. The pyruvate uptake by mitochondria was measured as described under Materials and Methods.

Under the same conditions conditions, 20 mM 2-phenylsuccinate, 1,2,3-benzenetricarboxylate, DL-*threo*-β-hydroxyaspartate or 2 mM mersalyl or *N*-ethylmaleimide (previously described as specific inhibitors of the dicarboxylate, tricarboxylate, α-oxoglutarate and phosphate carriers respectively) [16–18] do not significantly inhibit the swelling of yeast mitochondria in ammonium pyruvate (data not shown).

Fig. 2 shows the uptake of [^{14}C]pyruvate by yeast mitochondria. The linearity of the double-reciprocal plot of total pyruvate uptake versus external concentration of pyruvate shows that saturation kinetics are observed in the presence as well as in the absence of α-cyano-3-hydroxycinnamate. From this plot, an apparent K_m value of 6.2 mM pyruvate and a saturating uptake of 9.8 nmol · mg^{-1} of protein (corresponding to an intramatrix concentration of 12 mM) were estimated. This figure also shows that α-cyano-3-hydroxycinnamate acts as a competitive inhibitor of the transport of pyruvate with an apparent K_i of about 1 mM.

Figs. 1C and 1D show that the mitochondria isolated from the cytoplasmic petite mutant IL126-6C (rho$^-$) and the neutral petite IL126-6C (rho$^-$n) exhibit an osmotic response to isotonic ammonium pyruvate, similar to that obtained with fully functional mitochondria of the wild type yeast. The smaller extent of swelling response with mitochondria of the respiration-deficient mutants, particularly with mitochondria of the neutral petite, is probably due to a lower fraction of intact mitochondria in the mitochondrial preparation [19]. These results indicate that a pyruvate transporter exists in mitochondria of the petite mutants and that the presence of mitochondrial DNA and mitochondrial protein synthesis is not required for the biogenesis and the assembly of the pyruvate carrier in the mitochondrial membrane. The marked difference in the extent of the osmotic properties observed between the mitochondria isolated

from rho$^-$ and rho$^-$n petites is difficult to explain at the present time, but could indicate that mitochondrial DNA or RNA, both of which are present in rho$^-$ and absent in rho$^-$n, exert some general control on the permeability of the inner mitochondrial membrane.

Lactate transport

Passive swelling of wild type yeast mitochondria as well as of mitochondria of the cytoplasmic rho$^-$ and rho$^-$n petite mutants is observed in isoosmotic ammonium L(+)-lactate (Figs. 3A, 3C and 3D). The large amplitude swelling observed with the wild type mitochondria, as well as the small but consistent swelling observed with the rho$^-$ and rho$^-$n mitochondria is about 60–75 % inhibited by 20 mM α-cyano-3-hydroxycinnamate. These results are in agreement with those obtained by Halestrap et al. [6] in plasma membrane of human erythrocytes and with the results of Spencer and Lehninger [7] using ascites tumour cells, where α-cyano-3-hydroxycinnamate also inhibits the uptake of lactate. They differ however from the results obtained with rat liver mitochondria where no inhibition of lactate transport was observed. Our results suggest that the translocation of L(+)-lactate is carrier-mediated in the yeast mito-

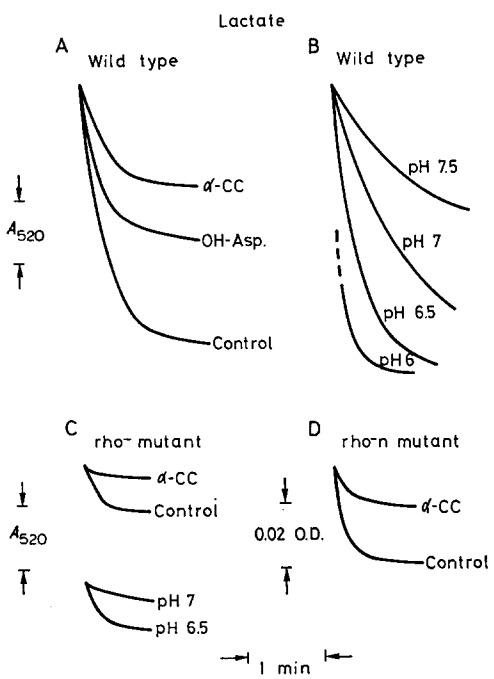

Fig. 3. Swelling of mitochondria in ammonium salt of L(+)-lactate. Swelling measurements are carried out as described in the legend of Fig. 1. (A) Swelling of fully functional mitochondria in 0.2 M ammonium lactate, preincubated in absence or presence of 20 mM α-cyano-3-hydroxycinnamate (α-CC) or 20 mM DL-*threo*-β-hydroxyaspartate (OH-Asp.). (B) Effect of pH change on swelling of mitochondria of the wild type strain. (C) Swelling of mitochondria of the respiratory deficient mutant (rho$^-$) effect of 20 mM α-cyano-3-hydroxycinnamate (α-CC) and effect of pH. (D) Swelling of the mitochondria of the neutral petite mutant (rho$^-$n) and effect of 20 mM α-cyano-3-hydroxycinnamate (α-CC).

chondrial membrane and that the carrier system is not a product of the mitochondrial genome. Figs. 3B and 3C also show that by increasing the pH, the rate of the lactate-induced swelling decreased markedly indicating, as for pyruvate, that the transport of lactate is proton or hydroxyl-coupled. This effect of pH together with the inhibition by α-cyano-3-hydroxycinnamate do not necessarily demonstrate that lactate is transported by the pyruvate carrier. Indeed, the swelling in ammonium lactate is also 50 % inhibited by 20 mM of DL-*threo*-β-hydroxyaspartate (Fig. 3A), a specific inhibitor of α-oxoglutarate transporter. In addition, the swelling in ammonium phosphate is about 50 % inhibited by 20 mM α-cyano-3-hydroxycinnamate (data not shown).

From this study, it is concluded that the transport of pyruvate and lactate across the yeast mitochondrial membrane are mediated by saturatable translocators inhibited rather specifically by α-cyano-3-hydroxycinnamate. Our results also indicate that the transport of pyruvate and L(+)-lactate are proton-coupled and that the transport system is not coded by mitochondrial DNA.

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