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PYRUVATE TRANSPORT IN TUMOUR-CELL MITOCHONDRIA

M.L. EBOLI[c], G. PARADIES[b], T. GALEOTTI[c] and S. PAPA[a]

[a] *Institutes of Biological Chemistry, Faculty of Medicine and* [b] *Faculty of Science, University of Bari, Bari and* [c] *Institute of General Pathology, Catholic University, Rome (Italy)*

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Summary

Tumour-cell mitochondria contain a pyruvate-transporting system exhibiting the same general properties as those described in rat liver mitochondria. The K_m for net pyruvate uptake in tumour-cell mitochondria is practically similar to that measured in rat liver mitochondria but the V is lower. This difference is also shown by swelling experiments. The possible implication of these observations in the context of lactate accumulation in tumour-cell is discussed.

Ehrlich ascites tumour cells exhibit, like other poorly differentiated neoplastic cells [1], high anaerobic and aerobic lactate production [2] in comparison with most mature normal cells.

The large aerobic accumulation of lactate in tumours has been ascribed to inadequacy of the shuttle mechanisms proposed for hydrogen-transfer from the cytosol to the mitochondrial compartment [1] and/or to impairement of the Pasteur effect, i.e. inhibition of glycolysis by oxygen-utilizing reactions (primarily mitochondrial oxidative phosphorylation [3]). It could also be due to insufficiency of mitochondrial pyruvate metabolism relative to the glycolytic activity of neoplastic cells.

Insufficiency of mitochondrial pyruvate metabolism might, in turn, be due to a lower content (or activity) of mitochondrial enzymes and/or a deficiency of pyruvate transport across the mitochondrial membrane.

Galeotti et al. [4,5] have presented evidence which would exclude insufficiency of hydrogen-transfer shuttle mechanisms in Ehrlich tumour cells. Insufficiency of the enzymes [4] and anion translocator [6] of the malate-aspartate shuttle in Ehrlich hyperdiploid cells as compared to the Ehrlich hyperdiploid Lettré mutant is apparently compensated for by high

activity of the α-glycerophosphate shuttle [4,7] and possibly of the fatty acid cycle [8].

It has recently been shown that pyruvate transport in rat liver mitochondria is mediated by a specific transport system [9,10]. Table I presents some general characteristics of pyruvate transport in ascites tumour-cell mitochondria. Pyruvate uptake was inhibited by α-cyanocinnamate (cf. ref.

TABLE I

INHIBITION BY α-CYANOCINNAMATE OF PYRUVATE UPTAKE BY TUMOUR CELL MITOCHONDRIA AND ANION-INDUCED EFFLUX OF MITOCHONDRIAL PYRUVATE

Expts.	Additions		Intramitochondrial level of pyruvate	
	In the preincubation medium	*In the incubation layer*	nmol	Δ
(a)	None	[^{14}C]Pyruvate	2.3	
	α-Cyanocinnamate	[^{14}C]Pyruvate	1.4	
(b)	[^{14}C]Pyruvate	None	7.9	
		Pyruvate	3.2	—4.7
		2-Oxobutyrate	3.2	—4.7
		Oxamate	3.4	—4.5
		Acetoacetate	4.3	—3.6
		Dichloroacetate	3.7	—4.2

Mitochondria were isolated from an Ehrlich hyperdiploid ascites tumour cell strain [7]. The initial rate of pyruvate uptake and efflux from mitochondria was measured by the centrifugation filtration technique [9,11]. Pyruvate uptake: Mitochondria (3.0 mg protein/ml) were preincubated at 22° C in: 150 mM sucrose, 30 mM Tris·HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM arsenite, 10 μg/ml oligomycin, 1.4 μg/ml rotenone, and 0.34 μg/ml antimycin A (final pH 7.2). After 4 min, the mitochondria were centrifuged through an incubation layer, at 4° C, containing 0.2 mM [^{14}C]pyruvate. Where indicated, 100 μM α-cyanocinnamate was present in the preincubation medium. Pyruvate efflux: Mitochondria (2.2 mg protein/ml) were preincubated 4 min in the standard reaction medium containing 2 mM [^{14}C]pyruvate. [^{14}C]Pyruvate loaded mitochondria were then centrifuged through a second incubation layer at 4° C containing various anions, as indicated, at 1 mM concentration.

10). [^{14}C]Pyruvate, accumulated by mitochondria, was rapidly released when unlabelled pyruvate or other substituted monocarboxylates were added to the external medium (cf. refs. 9,11). Citric acid-cycle intermediates, P_i and acetate were, on the other hand, ineffective in this respect. The monocarboxylate exchange-diffusion reactions were inhibited by α-cyanocinnamate (not shown).

Pyruvate uptake by tumour cell mitochondria followed saturation kinetics (Fig. 1). The rate of pyruvate uptake was enhanced by preincubation of mitochondria with 2-oxobutyrate (cf. ref. 11). This treatment decreased the K_m of pyruvate but left the V unchanged (see Fig. 1) (cf. ref. 11). The properties of pyruvate transport in tumour-cell mitochondria shown in Table I and Fig. 1 are quantitatively similar to those exhibited by this process in rat-liver mitochondria [10,11].

Tumour cell mitochondria underwent large-amplitude swelling when suspended in iso-osmotic solution of ammonium pyruvate (Fig. 2). This swelling of tumour-cell mitochondria was however slower and smaller than that exhibited, under the same conditions, by rat liver mitochondria (Fig. 2). Tumour-cell mitochondria, like rat liver mitochondria, did not undergo significant unspecific swelling when suspended in iso-osmotic mannitol plus sucrose or KCl.

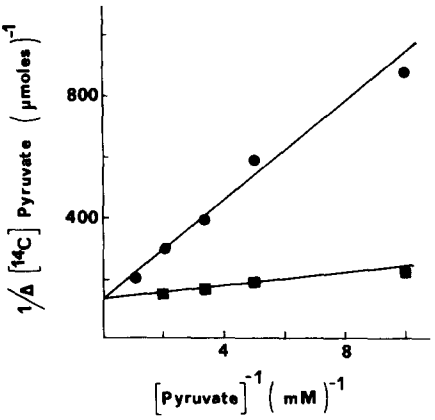

Fig. 1. Double reciprocal plot of [^{14}C]pyruvate uptake by tumour cell mitochondria. Mitochondria (2.8 mg protein/ml) were preincubated in the reaction medium described in the legend to Table I. After 4 min mitochondria were centrifuged through an incubation layer containing [^{14}C]pyruvate. The exposure time of mitochondria to the second incubation layer was 15 s [9]. ●——●, control; ■——■, 2 mM 2-oxobutyrate (added only in the preincubation medium).

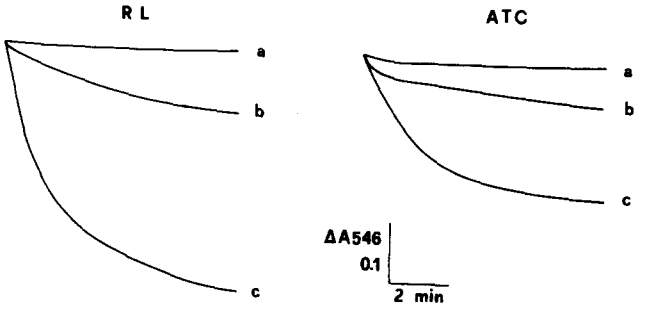

Fig. 2. Swelling of rat-liver (RL) and ascites tumour-cell (ATC) mitochondria suspended in isoosmotic ammonium pyruvate. Mitochondrial swelling was monitored at room temperature by measuring absorbance changes at 546 nm with an Eppendorf photometer. Mitochondria (0.35 mg protein/ml) were suspended in a medium containing: 10 mM Tris·HCl, pH 7.0, 1 mM EDTA, 0.4 μg rotenone/ml, 1.5 μg antimycin A/ml and trace (a), 225 mM mannitol, 75 mM sucrose and 0.1 mM EDTA; trace (b), 125 mM KCl; trace (c), 100 mM ammonium pyruvate. The reaction was initiated by addition of mitochondria to the medium.

The results presented show that there exists in Ehrlich ascites tumour cell mitochondria a pyruvate (monocarboxylate) translocator exhibiting the same general properties as those described in rat liver mitochondria [9,11].

The kinetic analysis of [^{14}C]pyruvate uptake (see below) and the swelling experiments show a lower activity (or content) of the pyruvate transport system in Ehrlich tumour cell mitochondria as compared to rat liver mitochondria. It should be pointed out that net pyruvate uptake by mitochondria is competitively inhibited by various substituted monocarboxylates which are produced by cell metabolism [11].

In the wild strain of Ehrlich tumour cells used in this study, lactate production in anaerobiosis is 3—10 times higher than in liver (see Table II). The specific activity of the mitochondrial pyruvate translocator is, on the other hand, 40% lower in Ehrlich cell mitochondria than in rat liver mitochondria. Since Ehrlich cells have only 6 mg mitochondrial protein per g wet weight, as

TABLE II

ANAEROBIC AND AEROBIC LACTATE PRODUCTION AND ACTIVITY OF PYRUVATE TRANSPORT AND UTILIZATION BY MITOCHONDRIA IN RAT LIVER AND EHRLICH HYPERDIPLOID ASCITES TUMOUR CELLS

	Lactate Production[a] (μmol·min^{-1}·g wet wt.$^{-1}$)		Pyruvate uptake by mitochondria				Pyruvate utilization by mitochondria (μmol·min^{-1}·g wet wt.$^{-1}$)	
	N_2	O_2	V (4°C) (nmol·min^{-1}·mg protein^{-1})	K_m (μM)	V^d (μmol·min^{-1}·g wet wt.$^{-1}$)	V^d(200 μM) (μmol·min^{-1}·g wet wt.$^{-1}$)	Oxidation[e]	Carboxylation[f]
Perfused liver	2.47	0.63						
Rat liver			20.1 ± 0.8[b]	640 ± 12[b]	10.36	4.28	2.86	2
Liver slices	0.83	0.34						
Ehrlich tumour cells	8.17	5.70	12.1 ± 0.4[c]	620 ± 18[c]	0.81	0.13	0.14	0.2

[a] The values reported for perfused liver are taken from ref. 12. Those for liver slices and ascites tumour cells from refs. 13 and 2, respectively. The factors responsible for the difference in lactate production in perfused liver and liver slices are not fully understood (ref. 12).

[b] See ref. 11.

[c] The values represent the mean of 8 experiments. The experimental conditions used to measure the kinetic parameters of pyruvate uptake were for both rat liver and tumour cell mitochondria those described in the legend to Fig. 1.

[d] The activity of pyruvate uptake per g wet weight is obtained on the basis of a content of 46 mg mitochondrial protein per g wet weight in liver cells [14] and of 6 mg in Ehrlich tumour cells as determined in this laboratory.

[e] The values for aerobic oxidation of pyruvate in rat liver and tumour cell mitochondria were obtained from measurements carried out at 1 mM pyruvate in the following reaction medium: 125 mM KCl, 20 mM Tris·HCl, 1 mM $MgCl_2$, 2 mM ADP and 2 mM potassium phosphate, pH 7.0.

[f] The values for pyruvate carboxylation in mitochondria from rat liver and ascites tumour cells are taken from refs. 15 and 16 respectively.

All the activities expressed per min and g wet wt. refer to a temperature of 38°C. The activity of mitochondrial pyruvate uptake at 38°C was calculated from the measurements carried out at 4°C, using a Q_{10} of 2 (Paradies, G., unpublished results).

compared to a content of approx. 50 mg in liver cells [14], the activity of the pyruvate translocator expressed on the basis of cell wet weight is one order lower in the tumour cells than in liver cells (Table II). From the data presented in Table II, it can be noted that in liver the activity of pyruvate uptake per g wet weight (at saturation as well as at 200 μM pyruvate, which is the physiological concentration in the cell [17]) and pyruvate utilization in mitochondria are of the same order as that of anaerobic lactate production. In Ehrlich tumour cells on the other hand, pyruvate uptake by mitochondria, as well as pyruvate utilization in mitochondria, are one order lower than anaerobic lactate production. It is therefore apparent that in Ehrlich tumour cells as compared to liver, there is a relative insufficiency of the systems involved in pyruvate uptake and utilization in mitochondria in bringing about the metabolic conversion of pyruvate produced by glycolysis. This insufficiency might be at least in part responsible for the high rate of lactate accumulation in Ehrlich tumour cells. In this respect, investigations of the conversion of glycolytic pyruvate to fatty acids in tumour cells would be of interest.

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