THE TRANSPORT OF PYRUVATE IN RAT LIVER MITOCHONDRIA

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1. Introduction

Recently numerous mitochondrial transporting systems have been described. Evidence for the existence in the inner membrane of rat liver mitochondria of translocators for adenine nucleotides [1], inorganic phosphate [2–6], citric acid cycle intermediates [3, 7–10] and amino acids [11] has been obtained. These systems appear to assure metabolic communication between the cytosol and the mitochondrial matrix. This paper concerns the transport of pyruvate in rat liver mitochondria. Evidence that the translocation of pyruvate across the inner mitochondrial membrane is directly coupled to hydroxyl ion counterflux or, which amounts to the same, to proton symport, is given. Results indicating that the transport of pyruvate across this membrane is mediated by a specific translocator are also presented.

2. Experimental

In the experiments of table 1 and fig. 1, the uptake of ^{14}C-pyruvate was followed by interrupting the incubation by rapid centrifugation of the mitochondria from the medium. In the experiments of figs. 2 and 3 and table 2, ^{14}C-pyruvate-loaded mitochondria were layered on the top of a second incubation layer and centrifuged through this into $HClO_4$. A discontinuous density gradient increasing towards the bottom of the centrifuge tube was made by the addition of dextran to the second layer. This was separated from the $HClO_4$, at the bottom of the tube by a layer of silicone oil. The exposure time of mitochondria to the second incubation layer (0.35 ml) was estimated to be about 15 sec by measuring the oxidation of β-hydroxybutyrate. The rate of this reaction was determined in separate controls [12]. The amount of ^{14}C-pyruvate remaining in the matrix space was calculated from the radioactivity in the $HClO_4$ extract. This was corrected for the ^{14}C-pyruvate present in the sucrose-permeable space plus adherent medium. Pyruvate was also determined enzymatically [13].

Table 1
Effect of pH and Pi on the uptake of ^{14}C-pyruvate by rat-liver mitochondria.

Additions	Pyruvate in the matrix (nmoles)		
	pH 6.5	pH 7.5	pH 8.5
None	59.0	35.8	21.3
Pi (1 mM)	34.4	26.1	13.7

Mitochondria (4.9 mg protein) were preincubated for 1 min in a reaction medium at various pH containing: 200 mM sucrose, 20 mM tris-HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 μg rotenone, 0.5 μg antimycin, 10 μg oligomycin and 1 mM arsenite. 2 mM ^{14}C-pyruvate was then added. Final volume, 1 ml. 2 min after the addition of ^{14}C-pyruvate, mitochondria were rapidly centrifuged from the medium.

3. Results

Table 1 shows the effect of the pH of the suspending medium and of added Pi on the uptake of ^{14}C-pyruvate by rat liver mitochondria. In this and the following experiments, mitochondria were preincubated with arsenite, rotenone (plus antimycin) and oligomycin, before adding pyruvate. This served to prevent energy supply and pyruvate oxidation. The increase of the pH of the medium inhibited the up-

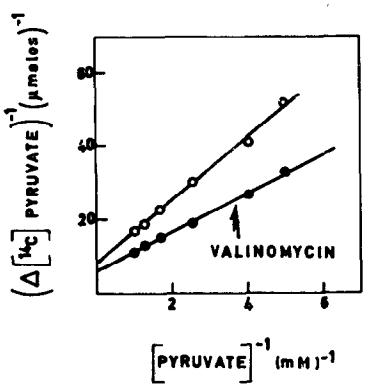

Fig. 1. Lineweaver-Burk plot of the uptake of ^{14}C-pyruvate, in the presence and the absence of valinomycin, by rat liver mitochondria. Mitochondria (7.8 mg prot.) were preincubated for 1 min in a reaction medium containing: 15 mM KCl, 75 mM tris-HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM arsenite, 1.4 μg rotenone, 0.34 μg antimycin and 10 μg oligomycin. Final volume 1 ml. Final pH 7.5. After 1 min ^{14}C-pyruvate, 50 μM TMPD, 3 mM ascorbate and where indicated, 0.2 μg valinomycin were added. After 15 sec mitochondria were rapidly centrifuged from the medium.

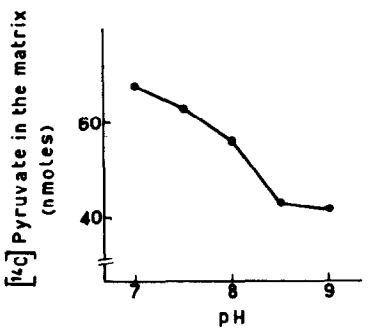

Fig. 2. Effect of the external pH on the efflux of ^{14}C-pyruvate from rat liver mitochondria. Mitochondria (5.7 mg protein), loaded with ^{14}C-pyruvate, as described in the legend to table 1, were centrifuged through a second incubation layer containing the same components of the preincubation mixture (except pyruvate). The pH of this layer was adjusted, in separate incubation, to the values shown in the figure.

take of pyruvate. The addition of Pi caused further inhibition. In the experiments of fig. 1, ascorbate and tetramethyl-*p*-phenylenediamine were added to the system so that energy could be supplied at the third phosphorylating site of the respiratory chain. The induction of K^+ uptake by valinomycin stimulated, under these conditions, the uptake of pyruvate (fig. 1). The uptake of pyruvate both in the presence and absence of valinomycin followed saturation kinetics (fig. 1).

In the experiment of fig. 2, mitochondria were loaded with ^{14}C-pyruvate and then transferred by centrifugation to a second medium free of pyruvate. During the few seconds of exposure to this medium, pyruvate moved out of the mitochondrial matrix. Increase of the pH of the second medium of incubation promoted the efflux of pyruvate. The efflux of ^{14}C-pyruvate, taken up by mitochondria during the first incubation, was also promoted by the addition of unlabelled pyruvate in the second incubation medium (table 2, fig. 3). The total level of intramitochondrial pyruvate determined enzymatically remained however unchanged. Thus intramitochondrial pyruvate moves out of the matrix in exchange–diffusion with extramitochondrial pyruvate. The plot of fig. 3 shows that the pyruvate–pyruvate exchange–diffusion followed saturation kinetics.

The experiment of table 2 shows that in short-time intervals added pyruvate did not exchange with intramitochondrial phosphate. Intramitochondrial pyruvate did not exchange with added Pi, malate, α-oxoglutarate or citrate. The pyruvate–pyruvate exchange was not inhibited by mersalyl, at a concentration which completely inhibited Pi transport; neither was it inhibited by butylmalonate which is a specific inhibitor of the dicarboxylate translocator [10, 14, 15].

4. Discussion

It has previously been demonstrated that the translocation across the inner membrane of rat liver mitochondria of inorganic phosphate, but not that of citric acid cycle intermediates, is directly coupled to a counterflux of hydroxyl ions (or to a proton symport) [6, 10]. The results presented here show that also the translocation of pyruvate is directly proton-coupled. The uptake of pyruvate by mitochondria is depressed by increasing the external pH or by adding inorganic phosphate to the medium (table 1); on the other hand, it is promoted by the valinomycin-induced energy-linked uptake of K^+ by mitochondria (fig. 1), a process which is accompanied by alkalinization of the intramitochondrial space [16, 17]. In short-time incubation at 0°, the efflux of pyruvate from the mito-

Table 2
Effect of anions and inhibitors on the intramitochondrial level of ^{14}C-pyruvate.

Additions	Amount in the matrix of		
	Pyruvate		Pi
	(nmoles)	Δ	(nmoles)
None	45.5		79.5
Pyruvate (1 mM)	21.3	-24.2	83.2
Mersalyl (100 μM)	40.9		153.7
Mersalyl+pyruvate	18.3	-22.6	150.2
Butylmalonate (0.5 mM)	39.8		99.9
Butylmalonate+pyruvate	12.1	-27.7	72.1
Pi (1 mM)	44.5		181.3
Pi+mersalyl	43.5		152.7
Malate (1mM)	44.1		
α-Oxoglutarate (1 mM)	45.3		
Citrate (1 mM)	41.2		

Mitochondria (5.8 mg protein) were loaded with ^{14}C-pyruvate as described in the legend to table 1. Where indicated, mersalyl and butylmalonate were added. ^{14}C-Pyruvate-loaded mitochondria were centrifuged through a second incubation layer containing the same components as the preincubation mixture (except ^{14}C-pyruvate). This layer contained the anions and inhibitors as indicated in the table.

chondrial matrix is stimulated, as is that of inorganic phosphate, by increasing the pH of the medium (fig.2). It should be recalled that under the same experimental conditions there is no effect of an external pH increase on the intramitochondrial level of citric acid cycle intermediates [10].

Intramitochondrial pyruvate moves of the mitochondrial matrix in exchange–diffusion with extramitochondrial pyruvate (fig. 3, table 2). The pyruvate–pyruvate exchange (fig. 3), as well as the respiration-linked uptake of pyruvate, either in the presence or absence of valinomycin-induced K^+ uptake (fig. 1) follow saturation kinetics. These findings provide evidence that the transport of pyruvate across the inner mitochondrial membrane is mediated by a translocator.

There is no direct exchange of pyruvate with inorganic phosphate, malate, citrate or α-oxoglutarate. Furthermore, the pyruvate–pyruvate exchange is not inhibited either by mersalyl or by butylmalonate (table 2). This shows that a pyruvate translocator is different from those mediating the transport of inorganic phosphate and citrate acid cycle intermediates.

Electron flow along the respiratory chain can be

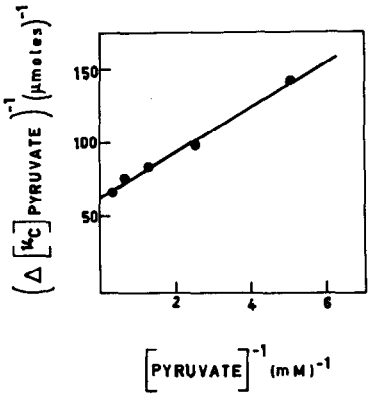

Fig. 3. Lineweaver-Burk plot of the exchange–diffusion of intramitochondrial pyruvate with extramitochondrial unlabelled pyruvate in rat-liver mitochondria. Mitochondria (6.3 mg protein), loaded with ^{14}C-pyruvate as described in the legend to table 1, were centrifuged through a second incubation layer containing the same components of the preincubation. This layer contained unlabelled pyruvate at different concentrations.

accompanied by proton ejection in the extramitochondrial phase [3, 18]. The translocation of pyruvate and phosphate across the mitochondrial membrane is proton coupled. Thus a pH difference, established across the membrane by respiration, can drive accumulation by the mitochondria of pyruvate – which is the most important natural respiratory substrate for mitochondria – and of inorganic phosphate. By means of this feedback mechanism an increased energy expenditure can be automatically compensated by replenishment of substrates at the site where they are utilized [19].

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