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ANION TRANSLOCATORS IN RAT-HEART MITOCHONDRIA

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1. Introduction

At least seven different anion translocators have been demonstrated in rat liver mitochondria [1, 2]. These translocators may be distinguished from each other by their specificity for substrates and their differential sensitivity to inhibitors [3]. Most anions enter the mitochondria in exchange for internal anions [4] (for a review, see [2]). A one to one stoichiometry has been found for the exchanges catalysed by the phosphate [5], the dicarboxylate [6], the oxoglutarate [6] and the tricarboxylate [6] translocators.

Tricarboxylate anions penetrate through the liver mitochondrial membrane in exchange either for intramitochondrial malate or for other intramitochondrial tricarboxylate anions [7, 8].

In liver mitochondria the dicarboxylate translocator catalyses not only a dicarboxylate/phosphate exchange, but also a dicarboxylate/dicarboxylate exchange [9]. The latter exchange may be effected by different translocators as proposed by Meijer and Tager [10] and Robinson et al. [3]. The phosphate moves across the mitochondrial membrane either via the phosphate translocator in exchange for hydroxyl ions, or via the dicarboxylate translocator in exchange for dicarboxylate ions.

Sulphydryl-binding reagents such as the mercurials [11, 12] and *N*-ethylmaleimide [13] inhibit the movement of phosphate. *N*-ethylmaleimide blocks the movement of phosphate via the phosphate translocator [14] and mersalyl via both the phosphate and dicarboxylate translocators [15]. The movement of dicarboxylates is also sensitive to mersalyl but the dicarboxylate/phosphate exchange may be completely blocked at concentrations that inhibit the dicarboxylate/dicarboxylate exchange partially [3, 10].

2-Oxoglutarate crosses the rat-liver mitochondrial membrane in exchange for dicarboxylate ions such as L-malate or malonate [16].

The purpose of this paper is to describe some of the properties of the anion translocators of heart-muscle mitochondria and to show that it is possible to dissociate the activity of the oxoglutarate translocator from those of the other translocators. The properties of the oxoglutarate translocator will be described in detail elsewhere. In addition, the present paper confirms the observation made by England and Robinson that the tricarboxylate translocator has little activity, if any, in heart mitochondria [17].

2. Materials and methods

Special reagents were obtained from the following sources: 2-^{14}C-malonic acid, 1,5-$^{14}C_2$-citric acid, $^{14}C_6$-sucrose, tritiated water, and ^{32}P-phosphoric acid (The Radiochemical Center, Amersham, England); oligomycin and rotenone (Sigma Chemical Company, Saint-Louis, Mo., USA); mersalyl, acid form, and *N*-ethylmaleimide (Mann Research Laboratories, New York, USA).

Rat-heart mitochondria were prepared according to Tyler and Gonze [18]. The mitochondrial proteins were determined by the biuret method as described by Beisenherz et al. [19] using serum albumin (Bovine Fraction V) as a standard. The standard incubation medium contained: 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris-Cl (pH 7.4), 11.25–22.5 mM mannitol and 3.75–7.5 mM sucrose (both derived from the stock mitochondrial suspension). Experimental details are given in the legends of the figures and tables.

3. Results

L-Malate and phosphate enhance the permeability of the rat-liver mitochondrial membrane for citrate [9]. Table 1 shows that in rat heart labelled citrate did not penetrate even in the presence of added L-malate or L-malate plus phosphate. The labelled citrate did not occupy more than the sucrose-permeable space and the concentration of the internal citrate (generated during the preincubation) remained constant; therefore, no citrate/citrate exchange occurred at 25° . The small amount of external citrate must have diffused out of the mitochondria during the preincubation in the presence of fluoroacetate.

Heart mitochondria incubated with *cis*-aconitate did not produce 2-oxoglutarate in the presence of ADP, phosphate and arsenite, unless valinomycin was added (fig. 1); in the latter case however drastic swelling preceded 2-oxoglutarate formation. This effect of valinomycin was also found when malate was added to the incubation medium (not shown).

If mitochondria preloaded with labelled phosphate were incubated in the standard medium, a loss of accumulated ^{32}P-phosphate was observed unless mersalyl or *N*-ethylmaleimide were also added (table 2); this is presumably the result of an exchange for hydroxyl ions. If phosphate or malate, both unlabelled, were added to the incubation medium, a further loss of radioactivity was observed which was completely abolished by mersalyl but not by *N*-ethylmaleimide (table 2). The phosphate efflux in the presence of *N*-ethylmaleimide must therefore have been brought about by the dicarboxylate transloactor.

Mitochondria about preloaded with ^{32}P-phosphate and incubated at 4° exchanged their labelled phosphate for external phosphate, but not for external malonate (fig. 2); this exchange was inhibited by *N*-ethylmaleimide. Similar experiments showed that internal labelled malonate (preloading carried out for 20 min

Table 1
Citrate/citrate exchange in rat-heart mitochondria.

Centrifugation after	(M) Citrate in the matrix space (μM)	(E) External citrate (μM)	$\frac{M}{E}$	Internal/external radioactivities	
				for citrate*	for sucrose
7.0 min	13,000	38	342	0.74	0.63
8.5 min	12,750	35	364	0.80	0.77
10.0 min	12,500	40	312	0.74	0.72
11.5 min	12,000	42	286	0.71	0.68

Mitochondria (final concentration = 1.8 mg protein/ml) were preincubated for 2 min in a 5 ml standard reaction mixture plus 30 mM phosphate, 30 mM glucose, 2 mM ATP, 5 mM malate and 10 mM fluoroacetate. Then 5 mM pyruvate and excess hexokinase were added. At t = 6 min, 2 μg rotenone were added per ml to stop further formation of citrate, followed 10 sec later by addition of 1 μCi radioactive citrate, carrier free. At the time indicated, 0.8 ml samples of the suspension were withdrawn and centrifuged through a layer of silicone oil to permit the separate determination of intra- and extramitochondrial citrates and radioactivities. Temperature was 25°.

* This ratio should approach the figures given in the preceding column if the internal membrane were permeable to citrate.

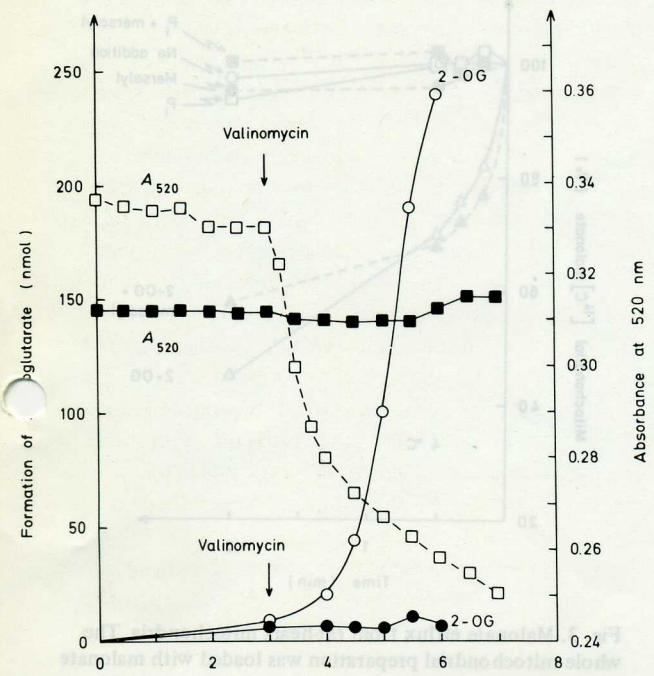

Fig. 1. Utilization of *cis*-aconitate by rat-heart mitochondria. The medium contained the standard components plus 20 mM phosphate, 3 mM ADP, 2 mM arsenite, 8 mM *cis*-aconitate, mitochondria (final concentration = 1.2 mg protein/ml for oxoglutarate formation, or 60 μg/ml for absorbance measurements), and valinomycin (final concentration = 0.05 μg/ml for oxoglutarate formation, or 2.5 ng/ml for absorbance measurements). 2-OG = 2-oxoglutarate.

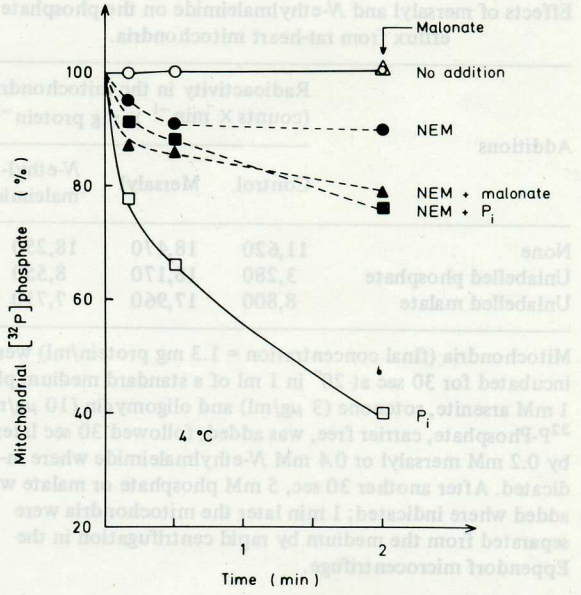

Fig. 2. Phosphate efflux from rat-heart mitochondria. Mitochondria (final concentration = 1.9 mg protein/ml) were first incubated at 4° in a standard medium plus 1 mM arsenite, rotenone (3 μg/ml) and oligomycin (10 μg/ml). ^{32}P-Phosphate, carrier free, was then added and followed, 30 sec later, by 0.4 mM *N*-ethylmaleimide (NEM) where indicated. After another 30 sec, 5 mM unlabelled phosphate or malonate was added (0 time of the graph) where indicated. The mitochondria were separated from the medium after further incubation at 4°, by rapid centrifugation in the Eppendorf microcentrifuge.

at 0°) did not exchange for external phosphate at 4°. Thus the activity of the dicarboxylate translocator is very much reduced, if not negligible, at low temperature, in both directions.

Malonate exchanged for phosphate at 20° in both directions, even in the presence of *N*-ethylmaleimide. It also exchanged for malonate, even in the presence of mersalyl. Since this malonate/malonate exchange operates also at 4°, this must be carried out by the 2-oxoglutarate translocator, the properties of which are illustrated in fig. 3.

The oxoglutarate translocator of rat liver mitochondria is insensitive to mersalyl [10]. This is also true in heart mitochondria.

Mitochondria preloaded with labelled malonate exchanged this anion for external 2-oxoglutarate, even at 4° (fig. 3) and this exchange was not significantly affected by mersalyl. Control experiments showed that the concentration of mersalyl used in the experiments of fig. 3 were sufficient to inhibit the malonate/phosphate exchanges (in both directions) at 20° completely.

4. Discussion

In tissues like liver, where fatty acid synthesis is an active process in the cytosol, intramitochondrial citrate is easily transported through the mitochondrial membrane by the tricarboxylate translocator. Citrate lyase in the cytosol splits citrate into oxaloacetate and the acetyl-CoA from which fatty acids are synthesized [20].

In heart, where fatty acid synthesis is exclusively

Table 2
Effects of mersalyl and *N*-ethylmaleimide on the phosphate efflux from rat-heart mitochondria.

Additions	Radioactivity in the mitochondria (counts × min^{-1} × mg $protein^{-1}$)		
	Control	Mersalyl	*N*-ethyl-maleimide
None	11,620	18,470	18,250
Unlabelled phosphate	3,280	18,170	8,550
Unlabelled malate	8,800	17,960	7,710

Mitochondria (final concentration = 1.3 mg protein/ml) were incubated for 30 sec at 20° in 1 ml of a standard medium plus 1 mM arsenite, rotenone (3 μg/ml) and oligomycin (10 μg/ml). ^{32}P-Phosphate, carrier free, was added, followed 30 sec later by 0.2 mM mersalyl or 0.4 mM *N*-ethylmaleimide where indicated. After another 30 sec, 5 mM phosphate or malate was added where indicated; 1 min later the mitochondria were separated from the medium by rapid centrifugation in the Eppendorf microcentrifuge.

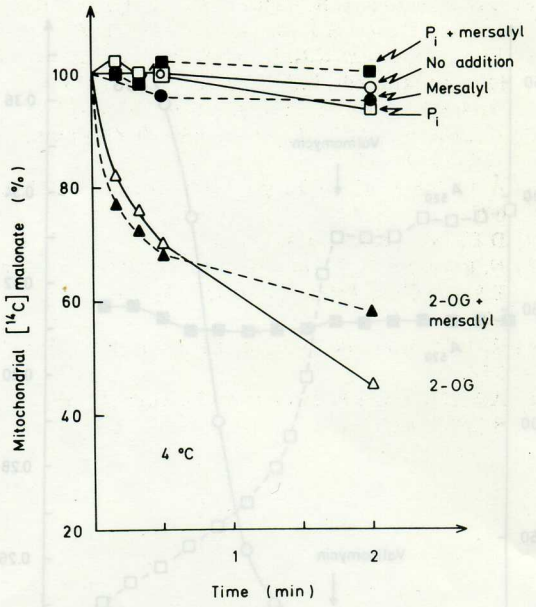

Fig. 3. Malonate efflux from rat-heart mitochondria. The whole mitochondrial preparation was loaded with malonate by incubation in a medium (20 ml) containing 225 mM mannitol, 75 mM sucrose, 0.05 mM EDTA, 5 mM malonate, and 20 mM Tris-Cl (pH 7.4) for 20 min at 0°. The mitochondria were then centrifuged and resuspended in 3 ml of the same medium containing 135 μM ^{14}C-malonate for 20 min at 0° before use. The mitochondria (final concentration = 0.93 mg protein/ml) loaded with labelled malonate were incubated at 4° in a standard medium plus 1 mM arsenite, rotenone (4 μg/ml) and 0.2 mM mersalyl where indicated. After 1 min, 0.5 mM 2-oxoglutarate or 1 mM phosphate was added (0 time of the graph) where indicated. The mitochondria were separated from the medium after further incubation, by rapid centrifugation in the Eppendorf microcentrifuge. 2-OG = 2-oxoglutarate.

mitochondrial [21] and where citrate lyase is not present [21, 22], no translocation of citrate is required for this purpose. Our results show the absence of an active tricarboxylate translocator in heart-muscle mitochondria (see also [17]).

Our results also show that heart-muscle mitochondria contain both phosphate and dicarboxylate translocators (contrast [24]). The first is sensitive to *N*-ethylmaleimide and to mersalyl, the latter to mersalyl only. The activity of the dicarboxylate translocator is negligible at low temperature.

Heart-muscle mitochondria, like liver mitochondria [10], contain an oxoglutarate translocator that is insensitive to mersalyl and active at 4°. This translocator catalyses an oxoglutarate/dicarboxylate as well as a dicarboxylate/dicarboxylate exchange.

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