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DICARBOXYLATE TRANSPORT IN THE INNER MEMBRANE MATRIX FRACTION OBTAINED FROM RAT LIVER MITOCHONDRIA

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Summary

Dicarboxylate transport was studied in the inner membrane matrix fraction (mitoplasts) and compared to that in intact rat-liver mitochondria from which the former was obtained.

It is concluded that, kinetics of dicarboxylate exchange measured in mitoplasts, are very similar to those observed with mitochondria. These results would indicate that the preparation technique preserves the integrity of the inner membrane and that neither the outer membrane nor the components of the peripheral space affect these results.

Quantitative kinetics are presently known for dicarboxylate transport in intact mitochondria [1—3] but not for mitoplasts.

The study of these transport mechanisms can be made on submitochondrial particles. However, the results obtained on this way for phosphate [4] or tricarboxylate transport [5] are different from those found in mitochondria.

As a first step, it appeared interesting to study the properties of the dicarboxylate carrier in inner membrane matrix fraction (mitoplasts) and to determine whether this preparation retains the same kinetic characteristics as intact mitochondria. This report gives quantitative results concerning kinetic constants of the uptake of various substrates linked to the dicarboxylate carrier. Other experimental parameters were also reported. Lastly, results obtained with mitoplasts are compared with those of mitochondria in identical experimental conditions.

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate.

Mitoplasts were prepared by digitonin treatment of mitochondria according to the technique of Schnaitman and Greenawalt [6]. Biological integrity was verified by electron microscopy and by the disappearance of kynurenine hydroxylase activity [7].

The kinetic of 0.034 mM malonate uptake in mitoplasts at 5°C is shown in Fig. 1A. It proceeds linearly with time, for approx. 20 s at a rate of 0.84 nmol · min^{-1}. Equilibrium is reached at 3 min of incubation. At this low concentration, malonate uptake follows a first-order reaction, as shown in Fig. 1B. The first-order constant is k = 1.15 min^{-1} and half-time is 35 s.

The rate of exchange reaction is influenced by temperature. From 0 to 13°C a straight line is obtained in a Arrhenius plot. Activation energy can thus be calculated as 19.4 kcal (81 kJ) and Q_{10} as 3.4. Optimum exchange rate is found near pH 7.0.

K_m and V values obtained for the various substrates studied with the mitoplasts and mitochondria, in the same experimental conditions, as shown in Table I. Malate and malonate have very close K_m, with malate slightly lower, while succinate is much higher. In addition, no significant difference is seen between the constant values in mitochondria and mitoplasts. With regard to the V, all three substrates, both in mitoplasts and in mitochondria, show very similar values. It should be noted, however, that the mitoplast preparation has a higher inner membrane content and that a V comparison is not valid unless the results are related to a constant membrane component such as cytochrome a, in the two preparations. In these conditions, the maximum rates obtained with the three substrates in the mitoplasts are very significantly lower than the rates

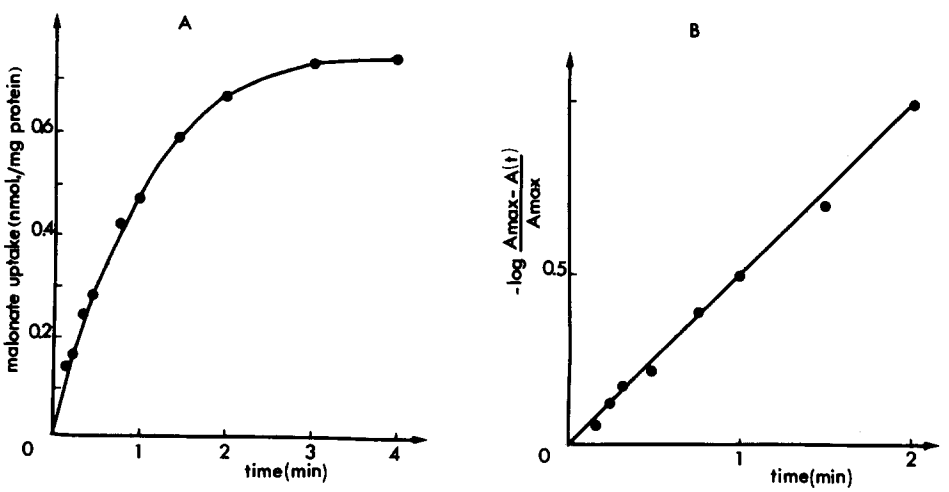

Fig. 1. (A) Kinetic measurement of malonate uptake. Mitoplasts (1.72 mg protein) were incubated for 20 s at 5°C in 0.56 ml medium consisting of 170 mM mannitol, 70 mM sucrose, 20 mM KCl, 10 mM Hepes, pH 7.0, in the presence of 1.5 μg oligomycin, 0.3 μg antimycin and 2 μg rotenone. Reaction started with addition of 0.034 mM [2-^{14}C]malonate, was stopped with 25 mM butylmalonate. After rapidly centrifuging the mitoplasts in an Eppendorf microcentrifuge, the radioactivity was measured in the pellets. A control is performed by adding the inhibitor before the substrate. The control value is deducted from the corresponding experiment. (B) Logarithmic plot of malonate exchange versus time assuming first-order type kinetics. A_{max} is the extent of maximum malonate uptake. $A(t)$ is the extent of malonate uptake at time t.

TABLE I

K_m AND V VALUES FOR THE UPTAKE OF MALATE, SUCCINATE AND MALONATE BY MITOPLASTS AND RAT LIVER MITOCHONDRIA

Experimental conditions were the same as in Fig. 1. The values given are the means ± S.E.; in parentheses: number of experiments.

Substrate	Mitochondria			Mitoplasts		
	K_m (mM)	V *	V **	K_m (mM)	V *	V **
Malate	0.062 ± 0.003(4)	8.1 ± 1.7	83.5 ± 17.5	0.059 ± 0.001(5)	7.0 ± 1.0	56.0 ± 8.0
Succinate	0.53 ± 0.03 (3)	9.2 ± 0.7	94.8 ± 7.3	0.51 ± 0.03 (4)	8.7 ± 0.6	69.6 ± 5.0
Malonate	0.075 ± 0.004(3)	6.7 ± 0.8	68.7 ± 7.8	0.083 ± 0.004(3)	6.5 ± 0.5	52.0 ± 4.2

* V expressed in nmol · min^{-1} · mg^{-1} protein.

** V expressed in nmol · min^{-1} · $nmol^{-1}$ cytochrome a. For mitochondria 1 mg protein = 0.097 ± 0.004 nmol of cytochrome a; for mitoplasts 1 mg protein = 0.125 ± 0.006 nmol of cytochrome a (six experiments).

obtained with mitochondria. The variations may be due to differences in the concentration of internal substrate which is exchangeable with dicarboxylate, preferentially inorganic phosphate [1]. Measurement of this compound gave the following results, expressed as concentration/μl of the matrix space: mitochondria, 31.6 nmol · μl^{-1}; mitoplasts, 19.7 nmol · μl^{-1}. (Matrix volumes are for mitochondria: 0.33 ± 0.02 (S.E.) μl · mg^{-1} protein or 3.40 μl · $nmol^{-1}$ cytochrome a and for mitoplasts 0.58 ± 0.05 (S.E.) μl · mg^{-1} protein or 4.64 μl · $nmol^{-1}$ cytochrome a in five experiments.) The variations in internal phosphate could account for the slight decrease in exchange rate observed in the mitoplasts since mitoplasts loaded with phosphate, to bring concentration to 54 nmol · μl^{-1}, have kinetic constants for malonate of 0.085 mM for K_m and 11 nmol · min^{-1} · mg^{-1} protein or 88 nmol · min^{-1} · $nmol^{-1}$ cytochrome a for V.

As with mitochondria [8] cations plays an important role in exchange reactions in mitoplasts; Ca^{2+} and Mg^{2+} increase the rate of dicarboxylate uptake. A more extensive study performed with Mg^{2+} shows that the penetration rate of malonate is optimum at 3 mM Mg^{2+}. At this concentration, the V is not changed but the K_m is lowered from 0.059 mM to 0.032 mM. Experiments performed with La^{3+} demonstrated that this cation, at concentrations as low as 0.1 mM, has a deleterious effect on the membrane. This fact suggests a greater fragility of mitoplasts; it is confirmed by the finding that at optimum Mg^{2+} concentration for the transport, all residual respiratory control has disappeared. This was demonstrated by Schnaitman and Greenawalt [6] and confirmed by us (Saint-Macary, M., Laine, M. and Foucher, B., unpublished results).

The data presented in this paper concerning mitoplasts are similar to those found for intact mitochondria and are characteristic of a carrier-catalysed transport. Compared to previous published results, the K_m observed for dicarboxylate exchange in mitochondria in this study, are the lowest reported. However, they remain close to the results obtained by Quagliariello et al. [2] and Palmieri et al. [3]. The variations observed would seem to be a result of experimental conditions: composition of the medium (type of buffer, ionic strength, cation composition) and temperature. Nevertheless, when comparing mitochondria and mitoplasts in identical experimental conditions, as done

herein, the results are indeed significant. Maximum velocities do not vary, whatever the dicarboxylate considered; however, even keeping in mind the relatively low temperature (5°C instead of 9°C), they do appear lower than those published by previous authors. Two factors may modify this rate: internal concentration of exchangeable anions and the difference of pH across the mitochondrial membrane. However, the use of mitochondria freshly prepared with inorganic phosphate did not lead to a significant increase in V.

With regard to kinetic constants of mitochondria and mitoplasts measured in the same conditions, close similarity is seen between K_m measured with the same substrates and for maximum rates expressed with relation to cytochrome *a* content when mitoplasts are preloaded with inorganic phosphate.

These finding demonstrate that mitoplasts retain the same dicarboxylate transport characteristics as intact mitochondria. The treatment required to remove the outer membrane does not modify the properties of the inner membrane and of the carrier. In addition, the outer membrane and the components of the peripheral space do not affect the exchange reaction.

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References

1 Passarella, S. and Quagliariello, E. (1976) Biochimie 58, 989—1001
2 Quagliariello, E., Palmieri, F., Prezioso, G. and Klingenberg, M. (1969) FEBS Lett. 4, 251—254
3 Palmieri, F., Prezioso, G., Quagliariello, E. and Klingenberg, M. (1971) Eur. J. Biochem. 22, 66—74
4 Rhodin, J.R. and Racker, E. (1974) Biochem. Biophys. Res. Commun. 61, 1207—1212
5 Prezioso, G., Stipani, I., Palmieri, F. and Quagliariello, E. (1977) FEBS Lett. 81, 249—252
6 Schnaitman, C. and Greenawalt, J.W. (1968) J. Cell Biol. 38, 168—175
7 Hayaishi, O. (1962) Methods Enzymol. 5, 807
8 Meisner, H., Palmieri, F. and Quagliariello, E. (1972) Biochemistry 11, 949—955