THE TRANSPORT OF MONOCARBOXYLIC OXOACIDS IN RAT LIVER MITOCHONDRIA

G. PARADIES and S. PAPA

Istituto di Chimica Biologica Facoltà di Scienze, Istituto di Chimica Biologica Facoltà di Medicina, Università di Bari e Centro di Studio sui Mitocondri e Metabolismo Energetico del C.N.R., Bari, Italy

Received 4 February 1975

1. Introduction

Recent investigations in our laboratory have shown that the transport of pyruvate in rat liver mitochondria is carrier mediated [1–4]. The kinetics parameters, specificity and sensitivity to sulphydryl blocking reagents of pyruvate translocation in mitochondria have been reported in a previous paper [5]. Further support to the existence of a pyruvate translocator in mitochondria has come from a recent paper by Halestrap and Denton [6], who reported inhibition of pyruvate transport in mitochondria by α-cyano-4-hydroxycinnamate.

Since evidence was obtained in our laboratory [4,5] that also acetoacetate is transported by the carrier involved in pyruvate translocation, we have now investigated the specificity of pyruvate carrier with respect to other oxoacids. It is shown here that besides pyruvate and acetoacetate, the pyruvate transporting system mediates the translocation of various monocarboxylic oxoacids. Thus this system may be named monocarboxylate translocator. The present results give some indications of the minimun structural requirement for a substrate in order to be transported by the monocarboxylate translocator.

2. Methods

Rat liver mitochondria were prepared as described by Myers and Slater [7]. 0.25 M sucrose was used for homogenization and washing. Pyruvate efflux from mitochondria was studied by the centrifugation filtration technique as follows (see also [5]). Mitochondria were loaded with [^{14}C] pyruvate in a reaction mixture containing: 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, 0.34 μg/ml antimycin. After preincubation, pyruvate loaded mitochondria were layered on the top of a second incubation layer at 4°C, and then spun down through this layer by rapid centrifugation. $HClO_4$ was immediately added to the mitochondrial pellet. The second incubation layer was of the same composition of the preincubation mixture, except the labelled pyruvate. The exchange-diffusion of mitochondrial [^{14}C] pyruvate with various oxoacids, was followed by adding the various anions to the second layer. Pyruvate was measured in $HClO_4$ extracts of the mitochondrial pellet and in the supernatant. The substrate content of the matrix space was calculated by correcting the amount in the mitochondrial extract with that in the sucrose permeable space plus adherent supernatant. This was determined with [^{14}C] sucrose. The mitochondrial level of pyruvate was determined either using [^{14}C] pyruvate or enzymatically [8]. Protein was determined by the usual biuret method.

3. Results

Table 1 illustrates the ability of various oxoacids to promote pyruvate efflux from [^{14}C] pyruvate loaded mitochondria. It can be seen that, at the concentration of 1 mM, besides unlabelled pyruvate and acetoacetate, also α-ketobutyrate, α-ketovalerate, α-ketoisovalerate, α-ketocaproate and α-ketoisocaproate, induced pyruvate efflux from mitochondria. In contrast γ-ketovalerate, ketomalonate and α-ketoglutarate were practically ineffective as pyruvate counteranions. Table 2 gives the kinetics parameters of the above exchange reactions

Table 1
Effect of various oxoacids on the intramitochondrial level of pyruvate in rat liver mitochondria

Additions (1 mM)	Intramitochondrial level of pyruvate (nmol)	Δ
None	31.5	
Pyruvate	12.9	18.6
Acetoacetate	16.9	14.6
α-Ketobutyrate	12.5	19.0
α-Ketovalerate	15.8	15.7
α-Ketoisovalerate	27.5	4.0
α-Ketocaproate	23.8	7.7
α-Ketoisocaproate	27.9	3.6
γ-Ketovalerate	31.5	0
Ketomalonate	31.1	0.4
α-Ketoglutarate	31.1	0.4

Mitochondria (7.2 mg protein) were preincubated in the sucrose reaction medium, described under Methods, in the presence of 2 mM [^{14}C]Pyruvate. Final pH 7.2. Final vol 1 ml. Temperature 20°C. After 2 min pyruvate loaded mitochondria were centrifuged, as described under Methods, through a second incubation layer containing the same components as the preincubation medium (except [^{14}C]pyruvate) and in addition the various oxoacids as indicated in the Table. For other experimental details see under Methods.

Table 2
Kinetics parameters of the exchange reactions of [^{14}C]pyruvate with various monocarboxylic oxoacids in rat liver mitochondria

	K_m (mM)	V_{max} (nmol/min/mg prot.) (4°C)
Pyruvate	0.113	11.5
α-Ketobutyrate	0.167	11.6
α-Ketovalerate	0.292	12.0
α-Ketocaproate	0.857	11.2
α-Ketoisovalerate	1.350	11.5
α-Ketoisocaproate	3.850	11.5
Acetoacetate	0.610	12.0

Mitochondria (7.1 mg protein) were preincubated in the sucrose medium described under Methods in the presence of 2 mM [^{14}C]pyruvate. Final pH 7.2. Final vol 1 ml. Temperature 20°C. After 2 min pyruvate loaded mitochondria were centrifuged through a second incubation layer containing the same components as the preincubation medium (except [^{14}C]pyruvate) and in addition different concentrations of unlabelled pyruvate or various oxoacids as reported in the table. For other details see the legend to table 1 and under Methods.

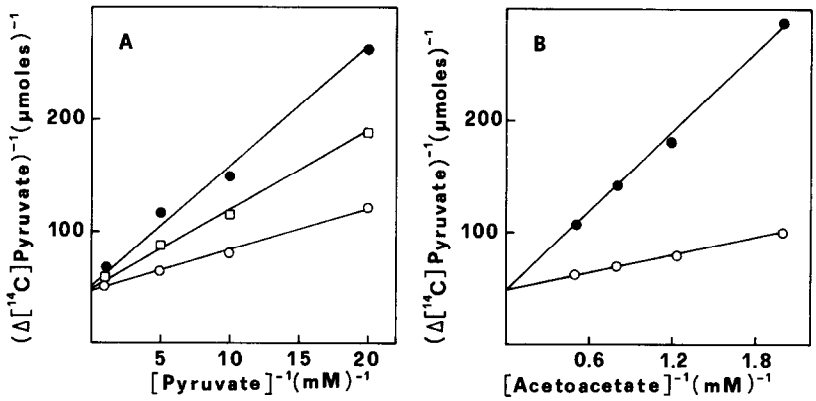

Fig.1. Double reciprocal plots of the effect of α-cyano-4-hydroxycinnamate on the pyruvate–pyruvate and pyruvate–acetoacetate exchange reactions in rat liver mitochondria. Mitochondria (8.0 mg protein) were preincubated in the sucrose medium described under Methods with 2 mM [^{14}C]pyruvate. Final pH 7.2 Final vol 1 ml. Final temperature 20°C. When present α-cyano-4-hydroxycinnamate (at the concentrations indicated below) was added to the preincubation medium 1 min after the addition of [^{14}C]pyruvate. 1 min after the addition of the inhibitor, pyruvate loaded mitochondria were centrifuged through a second layer containing the same components as the preincubation medium and in addition different concentrations of unlabelled pyruvate (A) or acetoacetate (B). (○–○) Control; (□–□) 50 μM α-cyano-4-hydroxycinnamate; (●–●) 100 μM α-cyano-4-hydroxycinnamate. For other experimental details see under Methods.

Table 3
Effect of α-cyano-4-hydroxycinnamate on the exchange reactions of [^{14}C]pyruvate with various monocarboxylic oxoacids

Additions (1 mM)	Intramitochondrial level (nmol) of pyruvate			
	Without α-cyano-4-hydroxycinnamate	Δ	With α-cyano-4-hydroxycinnamate	Δ
None	33.9		33.5	
Pyruvate	13.9	20.0	19.4	14.1
Acetoacetate	16.4	17.5	25.2	8.3
α-Ketobutyrate	14.3	19.6	19.5	14.0
α-Ketovalerate	16.3	17.6	23.9	9.6
α-Ketocaproate	20.3	13.6	29.8	3.7

Mitochondria (7.5 mg protein) were loaded with 2 mM [^{14}C]pyruvate as described in the legend to table 1. After 2 min, pyruvate loaded mitochondria were centrifuged through a second incubation layer containing the same components as the preincubation mixture (except [^{14}C]pyruvate) and in addition the monocarboxylic oxoacids as indicated in the table. When present α-cyano-4-hydroxycinnamate was added to the second incubation layer at the final concentration of 1 mM. For other details see the legend to table 1.

in mitochondria. Among all the oxoacids tested, pyruvate and α-ketobutyrate exhibited the highest affinity for the monocarboxylate translocator. The affinity of α-ketovalerate was higher than that of α-ketocaproate, besides both these oxoacids exhibited K_m values lower than those of α-ketoisovalerate and α-ketoisocaproate respectively. It will be noted, on the other hand, that all the exchange reactions exhibited the same V_{max}.

Fig.1 illustrates the kinetics of the inhibition of the pyruvate –pyruvate and pyruvate–acetoacetate exchanges by α-cyano-4-hydroxycinnamate. Both the exchanges were inhibited competitively by α-cyano-4-hydroxycinnamate.

The experiment reported in table 3 shows the effect of α-cyano-4-hydroxycinnamate on the exchange reactions of pyruvate with various monocarboxylic oxoacids. It can be seen that all the exchange reactions examined were inhibited by this compound. The extent of inhibition was, as expected for a competitive inhibitor, inversely related to the affinity of the counteranions for the translocator.

4. Discussion

This paper shows that mitochondrial pyruvate exchanges rapidly with a variety of externally added monocarboxylic oxoacids. These exchange reactions exhibit all the same V_{max}, but different K_m's, and are all inhibited by α-cyano-4-hydroxycinnamate. Thus they are apparently mediated by a common transporting system.

The present results allow us to specify a number of structural requirements of the monocarboxylate translocator. The first concerns the presence in the anion of both a carboxyl group and a carbonyl group and their relative position. In fact, as shown in table 1, the pyruvate transporting system accepts monocarboxylic acids with a carbonyl group in C_2 position. The presence of a carbonyl group in C_3 position, as in the case of acetoacetate, still permits translocation, although the affinity of the substrate for the transporting system decreases significantly (table 2). The presence of a carbonyl group in C_4 position, as in the case of γ-ketovalerate, does not permit translocation. Ketomalonate and α-ketoglutarate do not exchange with intramitochondrial pyruvate suggesting that the presence of more than one carboxyl group prevents transport by the monocarboxylate translocator.

The second requirement concerns the length of the carbon chain. In fact the affinity decreases as the chain length increases going from pyruvate to α-ketocaproate. Finally the affinity of the substrate for the transporting system depends upon the spatial arrangement of the carbon chain. In fact the exchange activity of α-ketoiso-

valerate and α-ketoisocaproate with intramitochondrial pyruvate is much lower than that of α-ketovalerate and α-ketocaproate respectively.

Our previous data [5] indicated that, at 1 mM concentration, the exchange of β-hydroxybutyrate or lactate with intramitochondrial pyruvate was very low as compared with that of the corresponding oxoacids. However at higher concentrations externally added β-hydroxybutyrate exhibited some exchange with mitochondrial pyruvate. This exchange followed saturation kinetics, however β-hydroxybutyrate exhibited a very long affinity for the transporting system, the Km being 5.9 mM (not shown). A movement of β-hydroxybutyrate on the mitochondrial pyruvate transporting system [9,10], would not have any physiological significance in the cell, in the well-fed state, since the concentration of β-hydroxybutyrate is, under these conditions of the order of 10^{-4} M [11]. However the transport of β-hydroxybutyrate on the monocarboxylate carrier, could occur at significant rates in starvation or in diabetes when the concentration of this substrate may rise to 10^{-3}–10^{-2} M [12].

Further studies are in progress in our laboratory to elucidate the specificity of the mitochondrial monocarboxylate translocator with the aim to understand the molecular mechanism by which these physiologically important anions are transported across the mitochondrial membrane.

Acknowledgements

The technical assistance of L. Gargano is gratefully acknowledged.

References

[1] Papa, S., Francavilla, A., Paradies, G. and Meduri, B. (1971) FEBS Lett. 12, 285–288.
[2] Paradies, G. and Papa, S. (1973) Boll. Soc. Ital. Biol. Sper. 49, 233–239.
[3] Paradies, G. and Papa, S. (1973) Abstr. Commun. 9th Int. Congr. Biochem. 288.
[4] Paradies, G. and Papa, S. (1974) Boll. Ital. Biol. Sper. 50, 338–344.
[5] Papa, S. and Paradies, G. (1974) Eur. J. Biochem. 49, 265–274.
[6] Halestrap, A. P. and Denton, R. M. (1974) Biochem. J. 138, 313–316.
[7] Myers, D. K. and Slater, E. C. (1957) Biochem. J. 67, 558–572.
[8] Bücher, T., Czok, R., Lamprecht, W. and Latzko, E. (1963) in: Methods in Enzymatic Analysis (Bergmeyer, H. U., ed.) pp. 253–259, Academic Press, New York.
[9] Mowbray, J. M. (1974) FEBS Lett. 44, 344–347.
[10] Land, J. M. and Clark, J. B. (1974) FEBS Lett. 44, 348–351.
[11] Berry, M. N., Williamson, D. H. and Wilson, M. B. (1965) Biochem. J. 94, 17c.
[12] Williamson, D. H., Lund, P. and Krebs, H. A. (1967) Biochem. J. 103, 514.