THE TRANSPORT OF OXALOACETATE IN RAT HEART MITOCHONDRIA

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1. Introduction

Oxaloacetate may be transported in rat liver mitochondria (RLM) by the oxoglutarate carrier, which catalyzes the exchange between OAA and oxoglutarate (or some other dicarboxylates) [1,2]. Although the transport of OAA in rat heart mitochondria (RHM) may have an important role in the transfer of reducing equivalents from the mitochondria to cytosol [3], only preliminary and contradictory results on OAA transport in heart mitochondria may be found. Whereas OAA causes efflux of intramitochondrial oxoglutarate [1], it is stated [4] that RHM are virtually impermeable to OAA. This paper examines the transport of OAA in RHM and presents evidence that OAA is transported by the oxoglutarate carrier at significant rates and with high affinity.

2. Materials and methods

Bathophenanthroline sulphonate, neocuproine, 8-hydroxy-quinoline, *p*-hydroxymercuribenzoate, amino-oxy acetic acid and malic enzyme were obtained from Sigma; phthalonic acid was prepared as in [5]. Other reagents were obtained as in [1,6,7].

Rat heart mitochondria were isolated and, when indicated, loaded with [^{14}C]oxoglutarate as in [1]. The loading procedure leads to an intramitochondrial concentration of oxoglutarate of 5–8 mM. The kinetics of oxaloacetate/oxoglutarate and oxoglutarate/oxoglutarate exchanges were measured by the 'inhibitor stop' method essentially as applied to RLM before [1,8]. [^{14}C]oxoglutarate-loaded mitochondria were incubated in 0.5 ml medium for 1 min under the condition specified in the legends. The assay was started by addition of unlabelled OAA or oxoglutarate and terminated 4–6 s later by rapid addition of 20 mM phenylsuccinate. During this period, the rate of exchange was constant within the limits of experimental error. After rapidly centrifuging the mitochondria in an Eppendorf microcentrifuge, the radioactivity in the pellets and supernatants was measured [9]. In order to calculate the rate of the exchange, the amounts of radioactivity and oxoglutarate present in the mitochondrial matrix and in the extramitochondrial phase prior the addition of the external substrate, were determined [1,8].

Changes in the redox state of intramitochondrial nicotinamide nucleotides were followed by fluorimetry [10] using an Eppendorf photometer (Model 1101 M) equipped with appropriate filters. The mitochondrial protein was determined by a modified biuret method [11]. Oxoglutarate was measured fluorimetrically as in [12]. For measuring the amount of oxoglutarate in the matrix the space available to ^3H_2O and [^{14}C]-sucrose was determined, in parallel experiments, as in [8,9].

Abbreviations: OAA, oxaloacetate; RHM, rat heart mitochondria; RLM, rat liver mitochondria

3. Results

In the extramitochondrial phase of RHM, suspended in an isoosmotic incubation medium, containing rotenone, the concentration of malate is neglegible, since the addition of malic enzyme does not reduce externally-added $NADP^+$ (not shown). If, however, $NADP^+$ and malic enzyme are added to the mitochondrial suspension after OAA (which per se

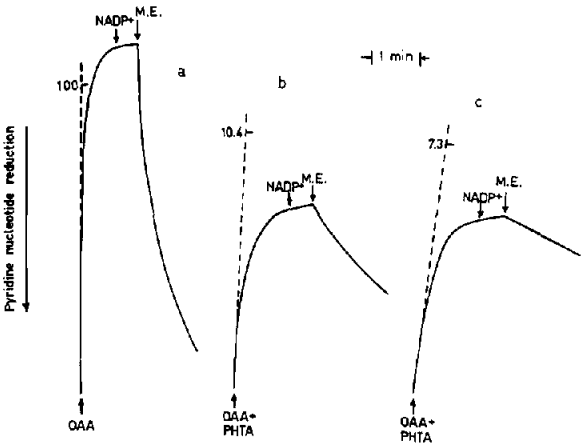

Fig.1. Uptake of OAA by RHM and appearance of malate in the incubation medium. RHM (0.5 mg protein) were preincubated for 2 min in 1.0 ml medium containing 0.2 M sucrose, 10 mM KCl, 1 mM $MgCl_2$, 20 mM Hepes–Tris pH 7.0 and 1 μg rotenone at 25°C. Where indicated, additions were as follows: 100 μM oxaloacetate (OAA), 30 μM in (b) and 100 μM in (c) phthalonate (PHTA), 250 μM $NADP^+$ and 0.1 units of malic enzyme (M.E.), previously dialyzed against 100 mM Tris–HCl buffer at pH 7.0. Nicotinamide nucleotide oxidation/reduction changes were followed fluorimetrically. (– – – –) Tangent to the initial part of the progress curve of the reactions. The numbers assigned to the tangents are their slopes in arbitrary units of scale divisions/min.

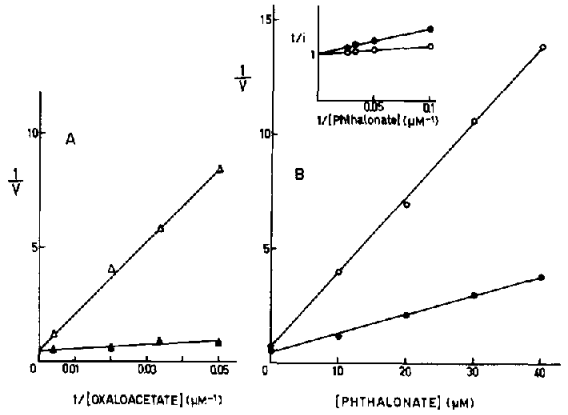

Fig.2. Kinetic analysis of the inhibition of oxaloacetate uptake by phthalonate using the double reciprocal plot (A) and the Dixon plot (B). RHM (0.8 mg and 1.1 mg protein in A and B, respectively) were preincubated for 2 min in 1.0 ml medium containing 0.2 M sucrose, 10 mM KCl, 1 mM $MgCl_2$, 20 mM Hepes–Tris, pH 7.0 and 1 μg rotenone at 25°C. In A, OAA was added at the concentrations indicated without (▲) or with 10 μM phthalonate (△). In B, OAA was added at 2 conc.: 50 μM (○) and 250 μM (●), and phthalonate at the concentrations indicated. The inset in B is a replot of the data, where $i = 1 - V_i/V$, and V_i and V are the rates of NAD(P)H oxidation in the presence and absence of phthalonate, respectively. The rate of NAD(P)H oxidation was followed fluorimetrically.

causes a fast oxidation of intramitochondrial NAD(P)H, a rapid and extensive reduction of pyridine nucleotides occurs (fig.1). Neither $NADP^+$ nor malic enzyme have any effect when added alone. These results imply the appearance of malate in the phase outside the mitochondria during the incubation of the mitochondria with OAA. A possible explanation of these results is that oxaloacetate is taken up in exchange of a small amount of oxoglutarate (or malate) via the oxoglutarate carrier. Inside the mitochondria, the OAA would be reduced to malate, which in turn could be exchanged for further OAA. The use of the oxoglutarate carrier by oxaloacetate is demonstrated by the sensitivity of oxaloacetate uptake to phthalonate, which is a specific inhibitor of the oxoglutarate carrier [13]. As shown in fig.1, phthalonate inhibits both the uptake of OAA and the efflux of malate. The nature of the inhibition on oxaloacetate uptake was studied by varying the oxaloacetate concentration at less than fully inhibitory amounts of phthalonate. The results, illustrated as a double reciprocal plot (fig.2A), suggest that the inhibition is competitive (oxaloacetate uptake K_m 20 μM; K_i 1 μM). This is further evidenced by a Dixon plot (fig.2B). The inset in which the reciprocal plots of the fractional inhibition (i) against inhibitor concentrations are reported, as advised [14], shows that linear functions are obtained which intersect the ordinate at unity, indicating that phthalonate is a completely competitive inhibitor with respect to oxaloacetate entry into RHM. In experiments not shown it was found that another inhibitor of the oxoglutarate carrier, i.e., phenylsuccinate [6,15], inhibits oxaloacetate entry into RHM in a competitive manner (K_i 15 μM). In agreement with the use of the oxoglutarate carrier, oxoglutarate was also shown to be a competitive inhibitor of OAA transport (K_i 15 μM). Vice versa, it was found that OAA inhibits the rate of oxoglutarate oxidation in RHM competitively, with K_i 40 μM.

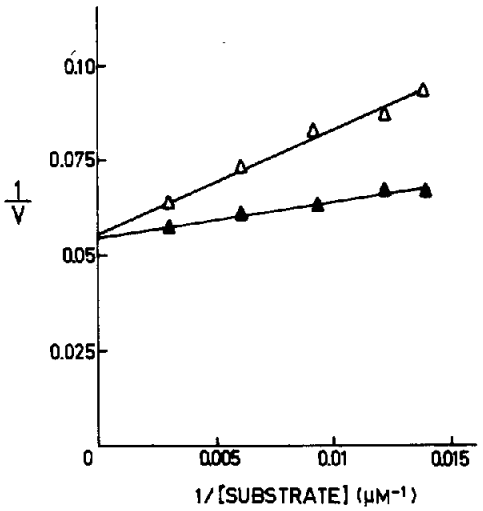

Fig.3. The dependence of the rate of the oxaloacetate/oxoglutarate and oxoglutarate/oxoglutarate exchanges on the external substrate concentration. Mitochondria loaded with [^{14}C]oxoglutarate (0.6 mg protein) were incubated at 8°C in 0.5 ml medium containing 0.2 M sucrose, 10 mM KCl, 1 mM $MgCl_2$, 20 mM Hepes–Tris, pH 7.0, 1 μg rotenone and 2 mM arsenite. The assay was started with oxaloacetate or oxoglutarate at the concentrations indicated, and stopped after 6 s by addition of 20 mM phenylsuccinate. (△) oxaloacetate/oxoglutarate exchange; (▲) oxoglutarate/oxoglutarate exchange. v is expressed as μmol/min/g protein. Other conditions were as in section 2.

Table 1
Effect of various anions, thiol reagents and metal-complexing agents on oxaloacetate uptake by heart mitochondria

Additions	Inhibition (%)
Malonate	45
Oxomalonate	60
Aminomalonate	3
Glutarate	28
Oxoglutarate	100
Glutamate	0
Aspartate	7
Phosphate	16
Sulphate	5
Thiosulphate	13
Mersalyl	100
p-Hydroxymercuribenzoate	33
N-ethylmaleimide	0
Bathophenanthroline	100
Neocuproine	44
8-Hydroxy-quinoline	42

RHM (0.6 mg protein) were added to 1 ml medium containing 0.2 M sucrose, 10 mM KCl, 1 mM $MgCl_2$, 20 mM Hepes–Tris, pH 7.0, 1 μg rotenone and 2 mM arsenite at 25°C. After 2 min, the reaction was started by the addition of 100 μM OAA. The additions, indicated in the table, were made simultaneously with OAA at 0.1 mM concentration, except NEM which was 0.56 mM. In the samples where the effect of aminoacids was tested, 2 mM aminooxyacetate was also included in the incubation mixture. The results are given as % inhibition NAD(P)H oxidation rate, measured fluorimetrically

In view of the lack of labelled oxaloacetate, the kinetics of oxaloacetate exchange were measured by following the efflux of [^{14}C]oxoglutarate from oxoglutarate-loaded mitochondria. For comparative purposes the kinetics of the oxoglutarate/[^{14}C]oxoglutarate exchange were also measured. The data from a typical experiment (fig.3) show that, with both substrates, linear functions are obtained, whose intersections with the ordinate are very close. This can be interpreted to show that V_{max} for the rate of oxaloacetate and oxoglutarate uptake is the same (approx. 18 μmol/min × g protein at 8°C). The slopes, however, are different, i.e., the K_m is higher for oxaloacetate than for oxoglutarate. In this experiment, the oxoglutarate K_m is 13 μM and OAA K_m is 50 μM.

The specificity of oxaloacetate transport in rat heart mitochondria was investigated by studying the effect of various anions on the rate of oxaloacetate uptake. As reported in table 1, dicarboxylates and oxodicarboxylates inhibit to a considerable degree the OAA-induced oxidation of intramitochondrial nicotinamide nucleotides. On the other hand, aminodicarboxylates and other anions such as phosphate and sulphate, do not cause significant effect. It should be noted that the aminodicarboxylates glutamate and aspartate were ineffective in the presence and in the absence of 2 mM aminooxyacetate, a competitive inhibitor of the mitochondrial aspartate-aminotransferase [16]. Table 1 also shows that the uptake of OAA by RHM is inhibited by the metal-complexing agents bathophenathroline, neocuproine and 8-hydroxy-quinoline, as well as by certain thiol reagents, like mersalyl and *p*-hydroxymercuribenzoate, but not *N*-ethylmaleimide. All these properties are distinctive features of the oxoglutarate carrier in RLM [6,17].

4. Discussion

The present results give evidence that the transport of OAA in RHM is mediated by the oxoglutarate carrier. Furthermore, by comparing the data reported in this paper with those obtained for RLM [1], it is clear that OAA is transported in RHM much more effectively than in RLM. Whereas in the latter the K_m for OAA transport (approx. 1 mM) is much higher than the concentrations of free OAA in the intact cell [18,19], the K_m for OAA transport in RHM (approx. 40 μM) is comparable with the cytosolic concentrations of OAA in heart (50 μM or more under some conditions [20]). The rate of OAA uptake in RHM extrapolated to 37°C with an activation energy about 20 kcal/mol is 760 μmol/min × g protein. It is apparent that the kinetic parameters of OAA transport in RHM are compatible with the possibility that an oxaloacetate influx and malate efflux via the oxoglutarate (either by a direct exchange or by a combined OAA/oxoglutarate and oxoglutarate/malate exchange) may account for the efflux of reducing equivalents from mitochondria in heart perfused with pyruvate [3]. Furthermore, in heart, the OAA/malate exchange would seem the only alternative for transfer of reducing equivalents to the cytosol, since the malate–aspartate cycle is unidirectional [21] and heart muscle does not contain sufficient pyruvate carboxylase and phosphopyruvate carboxykinase for the hepatic pyruvate–malate shuttle [22] to operate.

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