OXOGLUTARATE TRANSLOCATOR OF RAT-HEART MITOCHONDRIA: REGULATION BY ASPARTATE

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1. Introduction

The oxoglutarate translocator participates in the transfer of reducing equivalents from the cytosol to the mitochondrial respiratory chain via the malate–aspartate shuttle, by a cyclic process exchanging external malate for internal 2-oxoglutarate [1]. The catalytic components of the shuttle (the cytosolic and matricial glutamate–oxaloacetate transaminases and malate dehydrogenases, and the oxoglutarate and glutamate–aspartate translocators) act in sequence: one of the products is a substrate of the following step. Do allosteric effectors regulate the operation of the shuttle? This paper describes regulatory effects of aspartate on the oxoglutarate-translocator step of the shuttle working in the opposite direction (efflux of reducing equivalents from the mitochondrial matrix).

2. Materials and methods

Male fed rats were 200–250 g from an inbred strain of Wistar R/A Pfd f. Mitochondria from rat-heart ventricles were prepared according to [2]. The isolation medium contained: 225 mM mannitol, 75 mM sucrose, and 0.05 mM EDTA neutralized with 1 M Tris. Malate-loaded mitochondria were obtained after a 30 min incubation at 0°C in a medium containing 5 mM malate [3]; under such conditions internal aspartate was >5 mM. Malate-loaded but aspartate-depleted mitochondria were obtained by two successive incubations: a preliminary 30 min incubation at 0°C (in a medium containing 20 mM Tris–HCl buffer (pH 7.4), 1 mM arsenite, 5 mM 2-oxoglutarate, 184.5 mM mannitol, 61.5 mM sucrose and 0.041 mM EDTA) decreased the internal aspartate to 0.3–0.5 mM; the malate loading was obtained by a second incubation of 10 min at 20°C in the presence of 5 mM malate and 10 μM rotenone [3]. Both preparations were washed at 0°C in large volumes of incubation medium to obtain adequate ranges of internal malate concentrations without affecting the aspartate concentration. The incubation medium used for kinetic determinations contained 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl buffer (pH 7.4), 22.5 mM mannitol, 7.5 mM sucrose, 10 μM rotenone, 1 mM arsenite, 0.2 mM mersalyl and 6.67 μCi (247 kBq) ^3H_2O/ml.

Experimental timing, equipment and requirements for kinetic studies have been described in [3,4]. The matricial volume and the sucrose-accessible space were calculated from the content of mitochondrial pellets in ^3H_2O and [^{14}C]sucrose obtained by dual-labelling equilibration in parallel assays. Malate, oxoglutarate and oxaloacetate were determined as in [5–7]; aspartate was determined as in [8] and glutamate as in [9]. Mitochondrial protein was measured by the biuret method [10], using bovine serum albumin (fraction V) as a standard.

Abbreviations: OG, 2-oxoglutarate; Mal, L-malate; SD, standard deviation

3. Results and discussion

Malate-loaded (4.3 mM) mitochondria were incubated at 2°C in the presence of 54 μM oxoglutarate; the time course of the disappearance of external oxoglutarate and of the appearance of external malate was followed together with the variation in the amount of the internal oxoglutarate and malate and the total aspartate, glutamate and oxaloacetate (fig.1). The exit of oxoglutarate from the external compartment and the entry of malate into the same compartment showed a good one-to-one stoichiometry over the full time course. The internal oxoglutarate increased less than expected from the disappearance of external oxoglutarate, indicating a consumption of internal oxoglutarate. The internal malate decreased less than expected from the appearance of external malate, indicating a production of internal malate. Total glutamate increased while total aspartate decreased.

The data of table 1 were obtained after 3 min incubation: the changes of external oxoglutarate and malate show the one-to-one stoichiometry of the exchange whatever the initial concentration of inter-

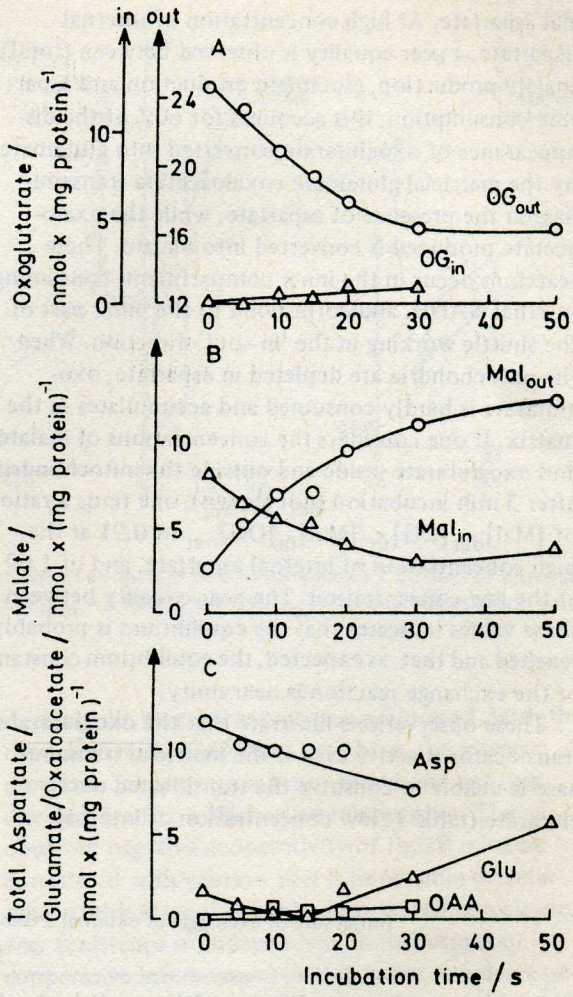

Fig.1. Variations of internal and external metabolites during the exchange of external 2-oxoglutarate for internal L-malate. Malate-loaded mitochondria contain 4.3 mM malate and 5.5 mM aspartate. Initiation and interruption of the incubation as in table 1: $[OG]_{out}$ = 54 μM; OAA, oxaloacetate; Asp, aspartate; Glu, glutamate.

Table 1
Changes in metabolites during the exchange of external 2-oxoglutarate for internal L-malate: Effect of internal aspartate

Internal conc. (mM)		Metabolites measured	Changes (nmol/mg protein)		
Aspartate	Malate		External	Internal	Total
5.72	3.79	Oxoglutarate	−9.8	+1	−8.8
		Malate	+9.8	−4.7	+5.1
		Aspartate			−5.4
		Glutamate			+5.1
0.17	3.06	Oxoglutarate	−5.5	+4.5	−1
		Malate	+5.4	−4.9	+0.5
		Aspartate			−0.2

The exchange is initiated by injection of 50 μM oxoglutarate in the incubation medium (0.5 ml) at 2°C and stopped by injection, simultaneously or after 3 min, of 0.2 ml of 80 mM phenylsuccinate (to measure the external changes) or of 2.5 M $HClO_4$ (to measure the total changes, external plus internal)

nal aspartate. At high concentration of internal aspartate, a near equality is observed between (total) malate production, glutamate production and aspartate consumption; this accounts for 60% of the disappearance of oxoglutarate converted into glutamate by the matricial glutamate–oxaloacetate transaminase in the presence of aspartate, while the oxaloacetate produced is converted into malate. These reactions occur in the inner compartment, consuming internal NADH, and correspond to the inner part of the shuttle working in the 'in–out' direction. When the mitochondria are depleted in aspartate, oxoglutarate is hardly consumed and accumulates in the matrix. If one considers the concentrations of malate and oxoglutarate inside and outside the mitochondria after 3 min incubation (not shown), one finds a ratio of $[Mal]_{out}[OG]_{in}/[Mal]_{in}[OG]_{out}$ of 0.91 at the high concentration of internal aspartate, and of 1.09 at the low concentration. The near equality between these values indicates that the equilibrium is probably reached and that, as expected, the equilibrium constant of the exchange reaction is near unity.

These observations illustrate that the oxoglutarate translocator is active even if the matricial transaminase is unable to consume the translocated oxoglutarate (table 1, low concentration of internal aspartate). Moreover, the initial rate of oxoglutarate translocation is not affected by aminooxyacetate, an inhibitor of the transaminase, in the presence of high or low levels of internal aspartate (not shown). The translocated oxoglutarate is therefore not sequestered in a catalytic complex consisting of oxoglutarate translocator and glutamate–oxaloacetate transaminase.

At 5 μM external oxoglutarate, added aspartate is without effect on the initial rate of translocation even if raised to 5 mM (table 2, expt. A,B). At a lower concentration of external oxoglutarate (0.59 μM) however (table 2, expt. C), 0.5 mM aspartate inhibits the initial rate of exchange by ~1/3rd, whether mersalyl is present or not (mersalyl has no effect of its own).

The depletion of internal aspartate from 6.9 to 0.42 mM affects the initial rate of the exchange of external oxoglutarate for internal malate (fig.2A). Internal aspartate is an activator at <4.5 mM malate and has no effect if it is ~4.5 mM (contrary to external aspartate which is an inhibitor at 0.59 μM external oxoglutarate and has no effect if its concentration is raised to 5 μM). In this range of malate concentrations, the apparent maximal rate is lowered by internal aspartate while the apparent affinity for internal

Table 2
Initial rate of exchange of external 2-oxoglutarate for internal L-malate: Effect of external aspartate

Expt.	OG_{out} (μM)	Asp_{in} (mM)	Mal_{in} (mM)	Asp_{out} (μM)	Initial rate ± SD	
					No mersalyl	0.2 mM mersalyl
					(pmol × s^{-1} × (mg protein)$^{-1}$)	
A	5	5.2	4.96	0		111 ± 2
				10		120 ± 6
				50		108 ± 6
			3.87	0		103 ± 11
				10		108 ± 10
				50		100 ± 5
			2.75	0		85 ± 5
				10		90 ± 5
				50		95 ± 7
B	5	4.3	6.65	0		188 ± 27
				5000		198 ± 8
C	0.59	10.5	1.69	0	20 ± 2	23 ± 2
				500	13 ± 2	13 ± 2

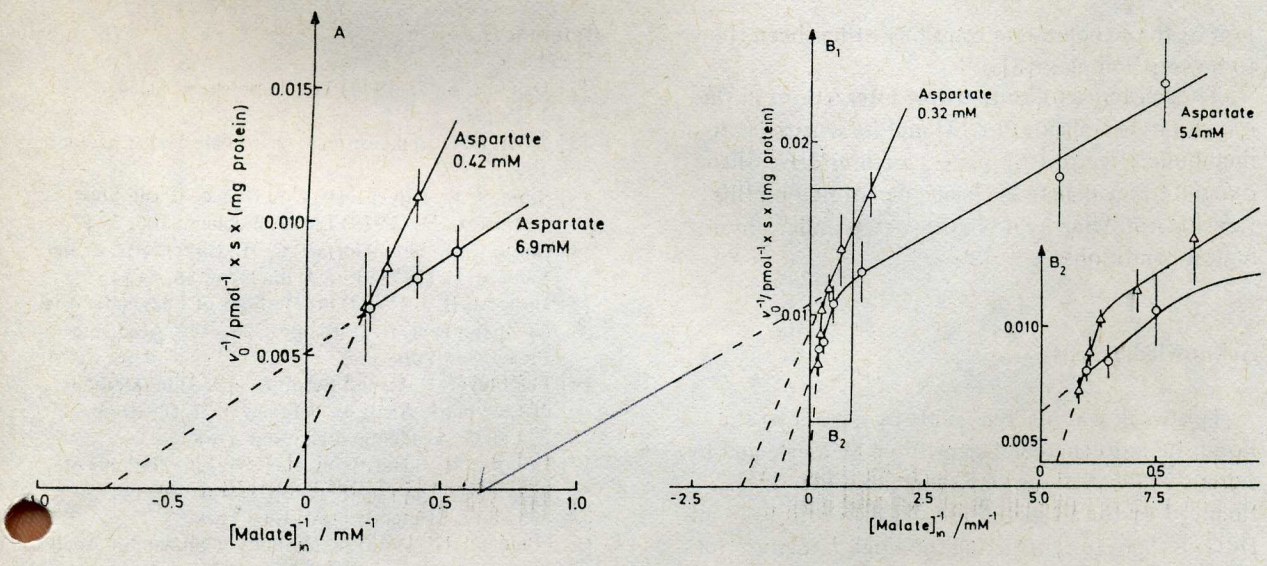

Fig.2. Initial rate of exchange of external oxoglutarate for internal malate as a function of internal [malate] : Effect of internal aspartate. (A) $[OG]_{out}$ = 5.18 μM and $[Mal]_{in}$ = 1.8–4.5 mM; (B) $[OG]_{out}$ = 5.08 μM and $[Mal]_{in}$ = 0.13–6 mM. Vertical bars represent the SD.

malate is increased (whereas external aspartate seems to decrease the affinity of the translocator for external oxoglutarate and to increase the apparent maximal rate). The resulting effect is a stimulation by internal aspartate at <4.5 mM internal malate; it should be an inhibition at >4.5 mM malate if linear extrapolation is permitted. (It has not yet been possible to increase internal malate to >4.5 mM while maintaining high internal concentrations of aspartate.)

The fact that the effects of external and internal aspartate are opposed could be related to the physiological function of the shuttle which consumes aspartate in the cytosol and produces aspartate in the matrix.

An attempt to extend the range of internal malate concentrations is reported in fig.2B. It confirms the preceding observation (fig.2A) but also shows an unexpected curvature indicating apparent negative cooperativity of internal malate in the presence of high and of low concentrations of internal aspartate. This differs markedly from the published curve (fig.4 in [3]) which showed slight upward curvature indicating apparent weak positive cooperativity.

As proposed in [3], the upward curvature obtained at that time could have been due to a modification of the concentration of an effector of the translocation; it might have been the result of a progressive decline of the internal aspartate concentration when the depletion in internal malate was performed. With the mitochondrial preparation used here, the internal aspartate concentration is not varied and yet this does not lead to a Michaelian relationship. The observed negative cooperativity of fig.2B must be considered with caution as it is impossible to determine at this stage if it can be interpreted in a classical way (existence of independent contributions or cooperative interactions) or if it is due to a decrease in the concentration of an unknown internal inhibitor accompanying the decrease of the internal malate concentration. Such artefactual deviations do not affect the kinetic saturation curve for the external substrate (fig.6 in [3]) as the internal conditions are kept constant.

4. Conclusion

These results indicate that the oxoglutarate translocator is regulated by internal and external aspartate in opposite ways. Regarding the initial rate of exchange between external oxoglutarate and internal malate, internal aspartate decreases the app. V_{max} and the app. K_m for the internal malate while external aspartate seems to increase the app. K_m and the app. V_{max} for the external oxoglutarate. This may be a simplified view of the aspartate effect as the behav-

iour of the oxoglutarate translocator has been shown to be very complex [3].

The existence of homotropic interactions in the oxoglutarate translocator [3] and its sensitivity to a metabolic effector (this paper) are indicative of the oxoglutarate–malate exchange performed by this translocator being a rate-limiting step under physiological conditions.

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