α-Oxoglutarate Carboxylation in Rat Kidney Mitochondria[1] (36744)

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Liver mitochondria, when incubated under reduced conditions *in vitro*, can catalyze the reductive carboxylation of α-oxoglutarate (1–11). This carboxylation occurs via the reversal of NADP-linked isocitrate dehydrogenase (2, 8, 12, 13) and yields primarily isocitrate and citrate (5, 9–12) which are equilibrated through aconitase (12). The $NADPH_2$ which is required for reduction of the enzyme-bound intermediate oxalosuccinate can be provided by many substrates (*e.g.*, succinate, β-hydroxybutyrate, fatty acids and α-oxoglutarate) but α-oxoglutarate is the most efficient donor (1, 9). In general, the reducing equivalents of substrate oxidation provide $NADH_2$ which can react with NADP via energy-linked transhydrogenase to form $NADPH_2$. The dismutation of α-oxoglutarate oxidation–carboxylation with the intermediate transhydrogenase step is shown in reactions [1–3]. If the mitochondria are incubated under anaerobic

[1] α-oxoglutarate + NAD + ADP + P_i → → succinate + $NADH_2$ + ATP + CO_2,

[2] ATP + $NADH_2$ + NADP + ADP → P_i + NAD + $NADPH_2$,

[3] α-oxoglutarate + CO_2 + $NADPH_2$ → (isocitrate ⇆ citrate) + NADP,

[4] Sum: 2 α-oxoglutarate → succinate + (isocitrate ⇆ citrate).

conditions or in the presence of malonate, succinate is the primary oxidative end product but if the respiratory chain is only partially inhibited as under "state 4" conditions (14) the succinate is oxidized and malate accumulates (9–11).

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The regulation of α-oxoglutarate carboxylation has been studied in liver mitochondria from normal (1–11) and diabetic (10, 11) rats but has not been studied in mitochondria from organs other than liver. In the present communication, α-oxoglutarate carboxylation is shown to occur in kidney mitochondria and a maximal rate for the carboxylation in kidney is projected from the mitochondrial results.

Materials and Methods. Animals. Male albino rats (250–300 g body wt) were used for all experiments. These rats were individually caged and fed Purina Chow pellets (Ralston Purina Co., St. Louis, MO), *ad libitum*.

Mitochondria. Mitochondria were isolated from rat kidney by the method of Johnson and Lardy (15) and supended in 0.25 *M* sucrose at an approximate concentration of 20 mg protein/ml. Protein was determined by the biuret procedure (16).

Experimental design. Mitochondrial incubations were performed in stoppered 25 ml Erlenmeyer flasks, at 37°, in a shaking water bath. Reactions were started by adding 0.5 ml of mitochondrial suspension to 2.5 ml of medium and stopped by adding 3 ml of 0.66 *M* perchloric acid. Processing of samples for analysis was done by the method of Walter, Paetkau and Lardy (3) except that samples were adjusted to pH 7.0 with phenol red as indicator. Tricarboxylic acid cycle intermediates and pyruvate were determined enzymatically by previously cited procedures (3, 17).

The media were prepared, as previously described (3, 9, 11, 18), by adding aliquots of reagents which were preadjusted to isoosmotic concentration. The volumes of the media were brought to 2.5 ml by addition of 0.7–1.2 ml of 0.15 *M* KCl. Milliosmolarities of the media were always between 280 and 310.

Results and Discussion. The α-oxoglutarate

oxidation–carboxylation dismutation in rat kidney mitochondria is demonstrated in Table I. Rat kidney mitochondria were incubated under chemically anaerobic conditions, produced by antimycin A, in the presence of malonate. Anaerobic conditions were used to maintain a highly reduced condition within the mitochondria by preventing the oxidation of $NADH_2$ via respiration. Malonate was included in the reaction mixtures to inhibit succinate dehydrogenase, thereby, preventing metabolism through the tricarboxylic acid cycle. At 20 m*M* malonate, succinate dehydrogenase was blocked and malate did not accumulate to detectable levels.

After 25 min incubation, considerable amounts of citrate accumulated and this citrate must have been synthesized via α-oxoglutarate carboxylation because the alternative synthesis through citrate synthase was indirectly blocked by the presence of malonate (*i.e.*, malonate prevented the oxidation of succinate to oxalacetate). The synthesis of citrate by condensation requires both oxalacetate and acetyl-CoA; acetyl-CoA might have been derived from the oxidation of endogenous precursors such as fatty acids, however, endogenous precursors of oxalacetate (*e.g.*, pyruvate and tricarboxylic acid cycle intermediates) were present at only barely detectable levels. The oxidation of α-oxoglutarate was not exactly coupled to the carboxylation as would be expected from reactions [1–3]; however, in this experiment no attempt was made to insure complete anaerobiosis and, therefore, it was possible that a small amount of α-oxoglutarate oxidation was coupled to respiration.

TABLE I. Reductive Carboxylation of α-Oxoglutarate by Kidney Mitochondria.[a]

Incubation time (min)	Metabolite changes (μmoles/3 ml reaction mixture)	
	α-Oxoglutarate consumed	Citrate accumulated
25	5.5 ± 0.2	1.99 ± 0.07

[a] The reaction mixtures (pH 7.4) contained 4 m*M* potassium disodium ATP, 10 m*M* $MgSO_4$, 6.7 m*M* potassium phosphate buffer (pH 7.4), 13.3 m*M* potassium triethanolamine buffer (pH 7.4), 6.7 m*M* potassium α-oxoglutarate, 20 m*M* $KHCO_3$, 20 m*M* potassium malonate, 1 μg antimycin A, 0.3% isopropanol, 32 m*M* KCl, 42 m*M* sucrose and 10 mg of mitochondrial protein. The values represent the mean ± SEM of 4 replicate determinations. The flasks were gassed for 1 min with a mixture of 95% N_2 + 5% CO_2.

TABLE II. The Metabolism of α-Oxoglutarate by Kidney Mitochondria as a Function of Incubation Time.[a]

Incubation time (min)	Metabolite changes (μmoles/3 ml reaction mixture)		
	α-Oxoglutarate consumed	Accumulated	
		Citrate	Malate
2.5	2.7 ± 0.3	0.20 ± 0.05	1.2 ± 0.01
5.0	4.4 ± 0.2	0.43 ± 0.03	2.3 ± 0.02
10.0	7.9 ± 0.1	0.89 ± 0.03	4.46 ± 0.04
15.0	11.1 ± 0.5	1.4 ± 0.2	6.73 ± 0.14
30.0	20.9 ± 0.5	2.8 ± 0.2	12.4 ± 0.3

[a] The reaction mixtures (pH 7.4) contained 4 m*M* potassium disodium ATP, 10 m*M* $MgSO_4$, 6.7 m*M* potassium phosphate buffer (pH 7.4), 13.3 m*M* potassium triethanolamine buffer (pH 7.4), 10 m*M* potassium α-oxoglutarate, 10 m*M* $KH^{14}CO_3$, 60 m*M* KCl, 42 m*M* sucrose and 8.0 mg of protein. The values represent the mean ± SEM of 4 replicate determinations.

Rat kidney mitochondria catalyzed the α-oxoglutarate oxidation–carboxylation dismutation under aerobic as well as anaerobic conditions when respiration was only partially inhibited as by the presence of ATP. When mitochondria from rat kidney were incubated in the controlled state[2] (Table II), citrate accumulated as under anaerobic conditions. Previous work has established that liver mitochondria under similar conditions synthesize citrate via reductive carboxylation (9, 11). However, that was not necessarily the case for kidney mitochondrial incubations where the ratio of α-oxoglutarate consumed:citrate accumulated was 3–4 times higher than that found with liver mitochondria. The poor correlation between α-oxoglutarate consumption and citrate accumulation was caused by oxidation via the respiratory chain since the

[2] The controlled state is used to refer to incubations under aerobic conditions with ATP added.

ratio of α-oxoglutarate consumed:citrate accumulated was greatly reduced by anaerobiosis. Respiration in the controlled state can be caused by ATPase activity or by uncoupled oxidative phosphorylation. Since respiration tends to lower the $NADH_2$:NAD ratio via oxidation of $NADH_2$ and, thereby, facilitate the conversion of malate to oxalacetate, it was necessary to show that kidney mitochondria in the controlled state synthesized citrate via reductive carboxylation and not via condensation.

In order to do this, mitochondria were incubated in the controlled state but with labeled bicarbonate ($H^{14}CO_3^-$). Fluorocitrate was added to inhibit aconitase. After 15 min incubation, citrate accumulated to 14.6% of the α-oxoglutarate used (Table III). Fluorocitrate at 0.017 and 0.07 m*M* reduced citrate accumulation by 67.4 and 68.9%, respectively, indicating that citrate was synthesized by α-oxoglutarate carboxylation. Measurements of $^{14}CO_2$ incorporation support this indication since fluorocitrate also inhibited $^{14}CO_2$ incorporation into citrate. The specific activity of the citrate was approximately 40 times higher than that of malate in the absence of fluorocitrate and 13 times higher in the presence of fluorocitrate. Thus, $^{14}CO_2$ was incorporated into citrate via α-oxoglutarate carboxylation and not via forward oxidation in the tricarboxylic acid cycle.

From the experiments presented above, it is not possible to postulate a role for α-oxoglutarate carboxylation in kidney metabolism; however, it is possible to project a limiting value for the rate of the carboxylation *in vivo*. Assuming that rat kidney contains 22 mg of N/g (19) and that 20% of this amount is mitochondrial (20) kidney could produce citrate via carboxylation at the rate of approximately 0.34 μmoles/g of kidney/min.[3]

Summary. Rat kidney mitochondria, incubated under aerobic or anaerobic conditions, catalyzed the reductive carboxylation of α-oxoglutarate. The rate of citrate accumulation was found to be an acceptable index of the rate of carboxylation. The projected maximal

[3] This value was the mean obtained from the 15 min control incubations of Tables II and III. It was assumed that 6.25 mg of protein contained 1 mg of N.

TABLE III. The Metabolism of α-Oxoglutarate by Kidney Mitochondria Incubated in the Controlled State in the Presence of Bicarbonate.[a]

Additions to system (m*M*)	Metabolite changes (μmoles/3 ml reaction mixture)				$^{14}CO_2$ in:			Sp act (%) of:	
	α-Oxoglutarate consumed	Accumulated		Total $^{14}CO_2$ incorporation	Fumarate	Malate	Citrate	Citrate	Malate
		Citrate	Malate						
None	13.0 ± 0.4	1.9 ± 0.1	7.80 ± 0.07	1.45 ± 0.07	0.025 ± 0.001	0.122 ± 0.006	1.22 ± 0.09	64.2 ± 5.4	1.56 ± 0.08
Fluorocitrate									
0.017	11.6 ± 0.48	0.62 ± 0.14	7.24 ± 0.24	0.647 ± 0.025	0.033 ± 0.002	0.205 ± 0.010	0.344 ± 0.024	55.5 ± 13.1	2.83 ± 0.17
0.07	11.9 ± 0.40	0.59 ± 0.17	8.09 ± 0.41	0.482 ± 0.023	0.040 ± 0.002	0.195 ± 0.010	0.180 ± 0.011	30.5 ± 9.0	2.41 ± 0.17

[a] The reaction mixtures (pH 7.4) contained 4 m*M* potassium disodium ATP, 10 m*M* $MgSO_4$, 6.7 m*M* potassium phosphate buffer (pH 7.4), 13.3 m*M* potassium triethanolamine buffer (pH 7.4), 6.7 m*M* potassium α-oxoglutarate, 10 m*M* $KH^{14}CO_3$, 60 m*M* KCl, 42 m*M* sucrose, and 9.9 mg of mitochondrial protein. The values represent the mean ± SEM of 4 replicate determinations. Incubation time was 15 min.

rate of the carboxylation which could occur in rat kidney was found to be 0.34 μmoles of α-oxoglutarate consumed/g of kidney/min.

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