ACTIVE OXIDATIVE DECARBOXYLATION OF MALATE BY MITOCHONDRIA ISOLATED FROM L-1210 ASCITES TUMOR CELLS

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Summary: Mitochondria from L-1210 mouse ascites tumor show a very high rate of oxidation of L-malate in comparison with mitochondria from normal tissues. They were found to contain large amounts of malic enzyme (E.C.1.1.1.39) catalyzing oxidative decarboxylation of L-malate to pyruvate. Malic enzyme in extracts of tumor mitochondria requires Mn^{2+} or Mg^{2+}, utilizes either NAD^+ or $NADP^+$ as electron acceptor, and shows positive cooperativity in binding of L-malate. These observations suggest that L-1210 tumor mitochondria actively convert excess tricarboxylate cycle intermediates and their precursors into pyruvate for further oxidation.

In a survey of metabolite transport and regulatory activities of mitochondria isolated from the ascites form of L-1210 mouse leukemia cells, it was noted that they differed markedly from most mammalian mitochondria in consuming oxygen at a high rate in the presence of L-malate alone. This observation suggested that oxaloacetate, the usual oxidation product of malate in mitochondria, was very rapidly removed. In this paper it is shown that these tumor mitochondria oxidize malate directly to pyruvate, in contrast to mitochondria from normal tissues which oxidize malate largely to oxaloacetate.

MATERIALS AND METHODS

L-1210 ascites tumor cells were grown in the peritoneum of mice of the BDF-1 strain. Approximately 5×10^6 cells in 0.1 ml of saline were inoculated; harvest was carried out four days later. Mitochondria were prepared from the washed ascites cells following treatment with the proteolytic enzyme Nagarse (6). Mitochondria from other sources were isolated using conventional techniques (7).

Sonic extracts were prepared as follows: 0.3 ml of a mitochondrial suspension (15 mg protein per ml) was diluted with 0.8 ml of 0.05 M potassium HEPES buffer pH 7.4, containing 0.6 mM $MnCl_2$ and 3 mM dithiothreitol. The

suspension was exposed to five 20-second bursts in an MSE ultrasonic disintegrator, with the sample cooled in an ice/salt mixture. After centrifugation at 100,000 g for 30 min the supernatant fraction was used for enzyme assay.

The composition of the medium used in each experiment is detailed in the appropriate legend. All experiments were performed at 30°.

RESULTS

The aerobic incubation of L-1210 tumor cell mitochondria with L-malate as the sole substrate results in rapid oxygen consumption and high respiratory control ratios (Fig. 1), much greater than observed with mitochondria from

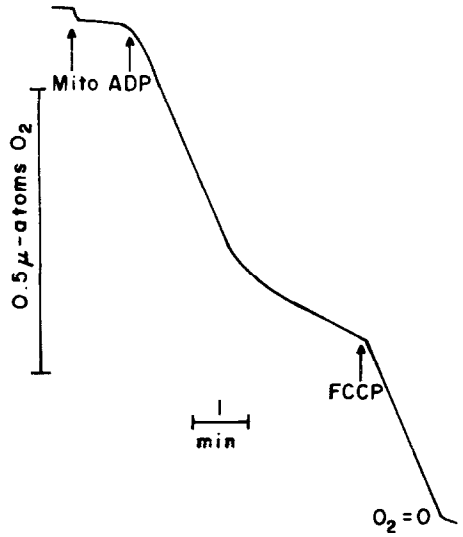

Fig. 1. Oxygen consumption by tumor cell mitochondria with L-malate as sole respiratory substrate. Mitochondria (0.61 mg protein) were added to a medium of 0.1 M KCl, 10 mM HEPES pH 7.1, 6.3 mM potassium phosphate pH 7.1, and 9.6 mM potassium malate. The total volume was 2.0 ml. Oxygen consumption was followed using a Clark type oxygen electrode in a water-jacketed chamber, closed except for a small capillary for making additions. ADP (approximately 1.2 μmoles) and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, 2 nmoles) were added as indicated.

some normal tissues (Table 1). Data in Table 1 also show that addition of cysteine sulfinate to transaminate with and thus remove any oxaloacetate formed produced no increment in rate of malate oxidation by the tumor mitochondria, but did enhance malate oxidation by the normal mitochondria. It is also seen

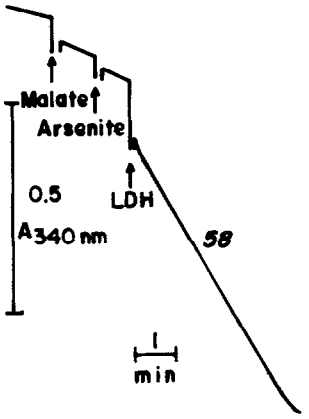

Fig. 2. The production of pyruvate during the oxidation of malate by tumor cell mitochondria. Mitochondria (0.25 mg protein) were added to 1 ml of a medium of 0.1 M KCl, 10 mM HEPES pH 7.1, 6.3 mM potassium phosphate pH 7.1, 0.8 mM ADP, and 0.1 mM NADH. Where indicated, 10 μmoles of malate, 2 μmoles of arsenite and 10 μg of lactate dehydrogenase (LDH) were added. The numbers indicate rates of NADH oxidation in nmoles/min/mg protein.

that the apparent affinity of the tumor cell mitochondria for malate is low compared to that of mitochondria from normal tissues. The apparent K_M of state 3 malate oxidation at this pH and ionic strength was found to be 1.4 mM; for normal rat liver mitochondria it is about 0.18 mM.

These findings suggested that in the tumor mitochondria malate undergoes oxidative decarboxylation to pyruvate, which is subsequently oxidized via the tricarboxylic acid cycle. The formation of pyruvate from malate by the L-1210 mitochondria was demonstrated by recording the oxidation of NADH in the presence of added lactate dehydrogenase and arsenite to prevent oxidative removal of pyruvate (Fig. 2). Under these conditions the rate of pyruvate production is approximately equivalent to the rate of oxygen consumption with malate, assuming that the pyruvate so formed is oxidized to completion via the cycle.

Extracts of L-1210 mitochondria prepared in 0.1% Triton X-100 caused rapid reduction of either NAD^+ or $NADP^+$ in the presence of malate, which was totally dependent on either Mg^{2+} or Mn^{2+}. Mn^{2+} gave higher rates and was used at 0.6 mM throughout; higher concentrations were slightly inhibitory. Comparison with Triton extracts of mitochondria from normal tissues revealed that

TABLE 1

A COMPARISON OF THE RATES OF MALATE OXIDATION BY MITOCHONDRIA FROM DIFFERENT TISSUES WITH THE ACTIVITY OF MITOCHONDRIAL MALIC ENZYME

Tissue of origin	Rate of oxygen consumption (ng-atoms O_2/min/mg mitochondrial protein)			Activity of malic enzyme (nmoles of $NAD(P)^+$ reduced/min/mg)		
	1. 2.4 mM malate	2. 9.6 mM malate	3. 9.6 mM malate plus 4.7 mM cysteine sulfinate	4. NAD^+ as acceptor	5. $NADP^+$ as acceptor	6. NAD^+ plus $NADP^+$
L-1210 ascites tumor cells	140	384	384	64	50	64
mouse heart	41	55	170	3	8	13
mouse liver	4.9	6.0	54	6.6	2.9	-
mouse kidney	52	58	112	5.2	9.6	12.6
rat brain	57	62	134	9.4	10.6	17.4

The activities of the malic enzymes were determined by adding mitochondria to 50 mM K HEPES pH 7.4 containing 0.1% Triton X-100, 10 mM malate, 0.6 mM $MnCl_2$, 3 mM dithiothreitol, 2 mM freshly prepared KCN pH 8 and 1 mM rotenone. Either 1 mM NAD^+, 1 mM $NADP^+$ or both were present; under these conditions the activity approximates to Vmax.

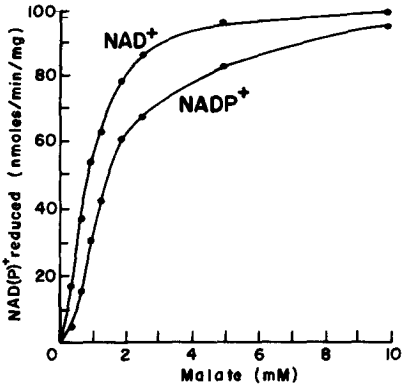

Fig. 3.

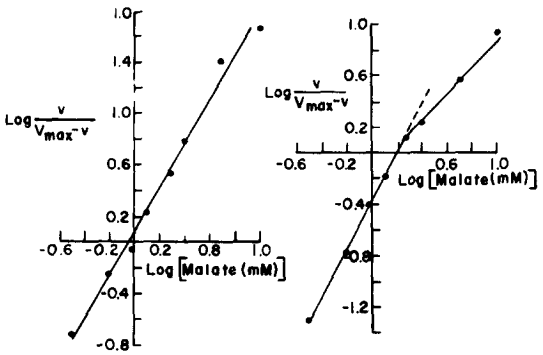

Fig. 4.

Fig. 3. Effect of malate concentration on the malic enzyme activity of sonic extracts of tumor cell mitochondria. The extracts were prepared as described in Methods. Enzyme (2.0 μl) was added to each cuvette, containing a total volume of 1 ml of 50 mM HEPES pH 7.4, 0.6 mM $MnCl_2$, 3 mM dithiothreitol, 3 mM KCN, 1 μM rotenone, 1.0 mM NAD^+ or $NADP^+$, and L-malate as indicated. The increase in $A_{340\ nm}$ was recorded and the rate expressed in terms of original mitochondrial protein.

Fig. 4. Hill plots derived from the data presented in Fig. 3. A, NAD^+ as coenzyme; B, $NADP^+$ as coenzyme.

malic enzyme activity is clearly highest in the tumor cell mitochondria (Table 1, columns 4, 5, and 6), which showed rates of NAD^+ reduction as high as 120 nmoles/min/mg of mitochondrial protein. There is a strongly positive correlation between the rate of malate oxidation (column 2) and the total activity of malic enzyme (column 6) in each mitochondrial type. The malic enzyme activity is adequate to explain the rate of malate oxidation by the tumor mitochondria, assuming six oxidative steps per malate molecule.

An exploratory investigation of the kinetics of the NADP-linked malic enzyme in extracts of L-1210 mitochondria (Fig. 3) suggested some cooperativity between malate binding sites, as found for the heart enzyme by Frenkel (4). A Hill plot of the data (Fig. 4) yielded a slope of 1.92. The NAD-linked activity likewise showed some cooperativity of malate binding (slope = 1.72), a property not reported previously. In these experiments essentially saturating concentrations of pyridine nucleotide were employed, approximately

10 times the K_M for the coenzymes. When the $NADP^+$ was decreased to 0.1 mM some decrease in cooperativity occurred (not shown). The reduction of both NAD^+ and $NADP^+$ is apparently caused by the same enzyme, as there is no increment in rate on adding NAD^+, for example, when the enzyme is already saturated with $NADP^+$. Moreover, Vmax is essentially the same with either coenzyme, as estimated from the data shown in Fig. 3.

DISCUSSION

The enzyme activity described in this paper, which reduced NAD^+ or $NADP^+$ in the presence of malate and Mn^{2+} or Mg^{2+}, is tentatively ascribed to malic enzyme, probably of the type E.C.1.1.1.39. The activity in the presence of NAD^+ cannot be ascribed to malate dehydrogenase (E.C.1.1.1.37) because of the absolute metal requirement and of the extent of reaction at neutral pH and low concentrations of malate and NAD. It is also unlikely that the NAD-linked activity is the sum of the action of malate dehydrogenase and a metal-requiring oxaloacetate decarboxylase, in view of the close similarity of the NAD- and NADP-linked activities and the lack of reactivity of malate dehydrogenase with NADP. In terms of nucleotide specificity the enzyme in the L-1210 mitochondrial extracts thus resembles that from Ascaris muscle mitochondria (8), rather than the NADP-linked enzyme from mammalian tissues (1,2,3,4) or the NAD-linked enzyme from mammalian heart (5).

The presence of an unusually active malic enzyme suggests that the tumor cell mitochondria may derive energy from the conversion of excess tricarboxylate cycle intermediates into pyruvate and subsequent oxidation of the pyruvate. If this is so, then the source of tricarboxylate cycle intermediates is of some interest. Oxidative deamination of amino acids is an obvious possibility.

Further work is required to determine whether the high activity of malic enzyme we have found in the mitochondria of the L-1210 tumor is a general property of mitochondria from all malignant cells.

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