THE ROLE OF MALIC ENZYME IN BOVINE ADRENAL CORTEX MITOCHONDRIA*

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The oxidative decarboxylation of malate to pyruvate and carbon dioxide was first observed by Moulder, Vennesland, and Evans (1945) and by Ochoa, Mehler, and Kornberg (1947). The possibility that the reaction might operate in the reverse direction to effect the fixation of carbon dioxide was suggested by Ochoa, Mehler, and Kornberg (1948). The enzyme catalyzing this reaction, malic enzyme (L-malate : NADP oxidoreductase (decarboxylating) 1.1.1.40), was purified from pigeon liver and characterized by Hsu and Lardy (1967). The enzyme was found to occur in the cytosol fraction of pigeon liver. Because of this intracellular location, Young, Shrago, and Lardy (1964) suggested that in liver and adipose tissue malic enzyme activity was important as a source of NADPH for lipogenesis.

Steroid mixed-function oxidases also require NADPH, but in the adrenal cortex three of these enzyme reactions are mitochondrial, namely the 11β-hydroxylase (Sweat, 1951; Brownie and Grant, 1954), 18-hydroxylase and cholesterol side-chain cleavage system (Psychoyos, Tallan, and Greengard, 1966; Halkerston, Eichhorn, and Hechter, 1961). The source of NADPH for these systems is not known, but evidence has been presented to suggest that the mixed-function oxidase electron transport chain of adrenal cortex mitochondria is linked to the main respiratory chain by an energy-dependent transhydrogenase,

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and that reducing equivalents generated during succinate oxidation can be coupled to this by reversed electron flow along the NADH-dehydrogenase portion of the respiratory chain (Harding and Nelson, 1966; Péron, McCarthy, and Guerra, 1966; Koritz, 1966; and Hall, 1967). In contrast, Grant (1956) suggested that the generation of NADPH for 11β-hydroxylation might be associated with malic enzyme activity in the mitochondria of the adrenal cortex.

The purpose of this communication is to show that bovine adrenal cortex contains two distinct malic enzymes, one in the cytosol and one in the mitochondria, and to present evidence that the mitochondrial malic enzyme activity is the source of NADPH for 11β-hydroxylation. The hypothesis that energy linked reactions occurring during succinate oxidation play a significant role in the generation of NADPH is not supported by the results presented in this paper.

Methods

Bovine adrenal cortex mitochondria were prepared as previously described (Cammer and Estabrook, 1967). Malic enzyme activity was assayed by means of NADPH formation using an Eppendorf fluorimeter with compensating voltage attachment as described by Estabrook and Maitra (1962). Alternatively, the increase in absorption at 340 mμ was observed using an Aminco-Chance dual wavelength split beam spectrophotometer. Oxygen uptake was determined using a Clark oxygen electrode (Clark, Wolf, Granger, and Taylor, 1963). Pyruvate estimation was by the method of Estabrook and Maitra (1962).

Results and Discussion

Differential centrifugation of a homogenate of bovine adrenal cortex revealed that malic enzyme activity was located in the mitochondria as well as in the cytosol. The total activity in the mitochondria was about one-fifth that in the cytosol, whereas the specific activity was about one-half that in the cytosol (25 mμ moles min^{-1} mg $prot^{-1}$ and 40 mμ moles min^{-1} mg $prot^{-1}$ respectively). The mitochondrial activity, however, was only manifest when the mitochondria were subjected to ultrasonication, which solubilized the enzyme,

thus indicating that the enzyme was located within the mitochondrion and was not merely absorbed on the outer surface, i.e. the mitochondrial activity was not due to contamination from the cytosol enzyme.

The mitochondrial and cytoplasmic malic enzymes of bovine adrenal cortex have been purified by ammonium sulphate fractionation and by chromatography on Sephadex G-100 and DEAE-cellulose. In this way, the mitochondrial enzyme was purified seventy fold. That the bimodal distribution of malic enzyme activity in the tissue was due to two distinct species of enzyme protein was shown by their chromatographic behaviour on DEAE-cellulose (Fig. 1). The cytosol enzyme behaved similarly to the pigeon liver enzyme of Hsu and Lardy (1967), but elution of the mitochondrial enzyme required a buffer of higher ionic strength.

Kinetic studies indicated a further difference between the cytoplasmic and mitochondrial enzymes. Measurements of the ratio of the maximum initial velocities for the forward and reverse reactions:

$$\text{malate} + \text{TPN}^{+} \underset{\text{reverse}}{\overset{\text{forward}}{\rightleftharpoons}} \text{pyruvate} + CO_2 + \text{TPNH} + H^{+}$$

indicated marked differences for the two enzymes. The ratio $(\text{Vmax})_{\text{forward}}/(\text{Vmax})_{\text{reverse}}$ for the mitochondrial enzyme was 105, whereas the ratio for the cytoplasmic enzyme was 2.5.

An interesting property of the mitochondrial enzyme was the strong inhibition observed in the presence of the respiratory uncouplers dinitrophenol and dicumarol. The inhibition by dicumarol was apparently competitive with respect to malate with a Ki of 15 μM. The supernatant enzyme was also inhibited by these reagents, but to a much smaller extent (Ki for dicumarol = 51 μM). The presence of a malic enzyme in the mitochondrial fraction inhibited by uncoupling agents raised the question of the validity of the interpretation that NADPH was generated during succinate oxidation via energy-linked reverse electron transport, since one of the experimental results, considered most critical for the evaluation of this pathway, is the inhibitory effect of

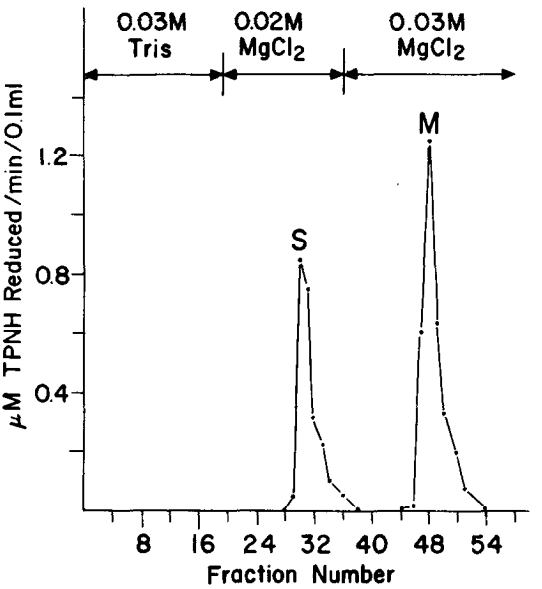

Figure 1. Chromatography of a mixed sample of mitochondrial and cytosol malic enzymes on DEAE-cellulose. 0.1 ml samples from each fraction were assayed in a final volume of 3 ml in the presence of 8 mM L-malate, 300 μM TPN, and 5 mM $MgCl_2$.
S - cytosol malic enzyme.
M - mitochondrial malic enzyme.

uncouplers on the succinate-supported 11β-hydroxylation of DOC (Guerra, Péron, and McCarthy, 1966).

Experiments were then carried out to measure pyruvate formation during malate- or succinate-supported 11β-hydroxylation of DOC by adrenal cortex mitochondria. Arsenite was added to the reaction medium to inhibit pyruvate oxidase activity, and rotenone to inhibit NADH dehydrogenase. As can be seen in Figure 2, the 11β-hydroxylation of DOC in the presence of succinate produced a stimulation of pyruvate formation as a result of malic enzyme activity. The rate of pyruvate formation was sufficient to provide all the NADPH required for the hydroxylation reaction, as measured by the stimulation of oxygen uptake. A similar situation was found if rotenone was omitted. Comparable results were obtained when malate was used as substrate. Thus bovine adrenal cortex contains, in addition to the normal cytoplasmic enzyme, a second malic enzyme located in

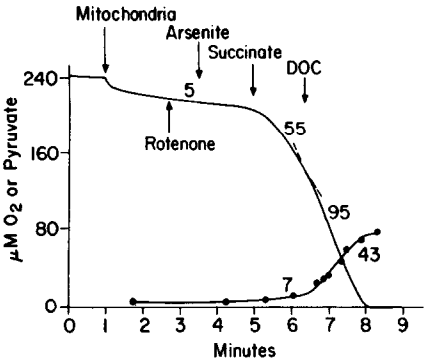

Figure 2. Oxygen uptake and pyruvate formation during succinate-supported 11β-hydroxylation of DOC. Oxygen uptake was measured in the chamber of a Clark oxygen electrode. In a parallel experiment samples were taken for pyruvate estimation as described in the text. Concentrations in the final mixture were: 8 mM succinate; 135 μM DOC; 2 mM arsenite; and 3 μg/ml of rotenone. Oxygen uptake is shown as a continuous line, pyruvate formation as solid circles. The numbers on the figure are rates of oxygen uptake or pyruvate formation in μM min^{-1}.

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the mitochondria, the function of which is to provide NADPH for mitochondrial mixed-function reactions. Previous conclusions regarding the role of an uncoupler-sensitive energy-dependent succinate-linked reduction of NAD^+, coupled with an energy-dependent transhydrogenase for formation of NADPH, need not be considered as an obligatory requirement for the mixed-function oxidase reaction.

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