ALLOSTERIC CHARACTERISTICS OF BOVINE HEART MITOCHONDRIAL MALIC ENZYME*

by

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Received April 20, 1972

SUMMARY. The mitochondrial isozyme of malic enzyme from bovine heart shows allosteric properties at low concentrations of malate and this characteristic can be modified by succinate, which does not affect the maximum velocity of the reaction. The cytoplasmic isozyme from the same tissue does not show any allosteric properties.

INTRODUCTION. A previous communication from this laboratory (1) has shown that two isozymes of malic enzyme (malate:$NADP^+$ oxidoreductase (decarboxylating) E.C.1.1.1.40) are found in bovine heart muscle. These isozymes are localized in different subcellular structures and it has been possible to separate them by chromatography on DEAE cellulose. By means of differential extraction or by preparation of isolated mitochondria it was also shown that one of the malic enzyme isozymes (Enzyme I) resides in the cytosol, while the second isozyme (Enzyme II) is contained in the mitochondrial space. Brdiczka and Pette (2) and Isohashi, et al., (3), have also shown the dual localization of malic enzyme in heart tissue from several species. These observations place heart muscle in the same category of adrenal cortex (2,4) and brain (5, 6), since in these tissues malic enzyme is found in both the cytosol and the mitochondria. Liver, adrenal medulla, and adipose tissue cells, on the other hand, appear to contain exclusively a cytoplasmic malic enzyme (2).

During the course of the studies with the partially purified isozymes of malic enzyme from bovine heart, it became evident that the mitochondrial isozyme presents clear allosteric characteristics, a property which is not apparent

* Supported by Grants AM-13275 from NIH and I-385 from the Robert A. Welch Foundation.

in the cytoplasmic isozyme. It is the purpose of this communication to show that the activity of the bovine heart mitochondrial malic enzyme does not follow Michaelis-Menten kinetics at low concentrations of malate and that succinate can alter this characteristic by affecting the cooperativity shown by malate, without affecting the maximum velocity of the reaction. No such phenomena are seen with the cytoplasmic isozyme.

METHODS AND MATERIALS. The cytoplasmic (E I) and mitochondrial (E II) isozymes from bovine heart were prepared and purified as described previously (1). In all the studies to be reported here, E II was obtained from previously isolated mitochondria and E I from a whole tissue homogenate prepared in 0.25 M sucrose to minimize mitochondrial breakage. Both enzymes were purified through the CM-Sephadex step as previously described (1). Further purification was obtained by passage through a 2.5 cm x 90 cm column of Bio-Gel 1.5 m, previously equilibrated with a solution containing 25 mM KCl, 10 mM N-morpholinopropane sulphonate, pH 7.5 and 1 mM 2-mercaptoethanol. The enzymes were eluted from the column with the same buffer. This step removed some contaminating proteins, while retaining the complete activity of malic enzyme, and resulted in a 2.5-fold increase in the specific activity of E I and a 3-fold increase in the specific activity of E II. After elution from the column, the active fractions were pooled and concentrated by means of an Amicon Diaflo ultrafilter, equipped with a PM-30 membrane. Retention in these cases was always better than 97%. The concentrated enzyme solutions were made 50% saturated with respect to ammonium sulfate by the addition of an equal volume of the saturated salt solution at 4° and pH 7.4. The mitochondrial malic enzyme is quite stable under these conditions, showing less than a 5% loss of activity per week if stored at 4°. The cytoplasmic isozyme appears less stable and a 10% - 15% loss of activity per week can be expected under the same condition

The method for the measurement of enzymic activity was essentially the same described before (1), except that all assays were performed at pH 8.0 instead of 7.5.

Succinic acid, L(-) malic acid, N-morpholinopropane sulfonic acid, and

$NADP^+$ were purchased from Sigma Chem. Co., St. Louis, Mo. Bio-Gel 1.5 m was obtained from Bio-Rad Laboratories, Richmond, Calif.

RESULTS AND DISCUSSION. The effect of varying the malate concentration on the reaction rates of both isozymes of malic enzyme, is shown in Figure 1.

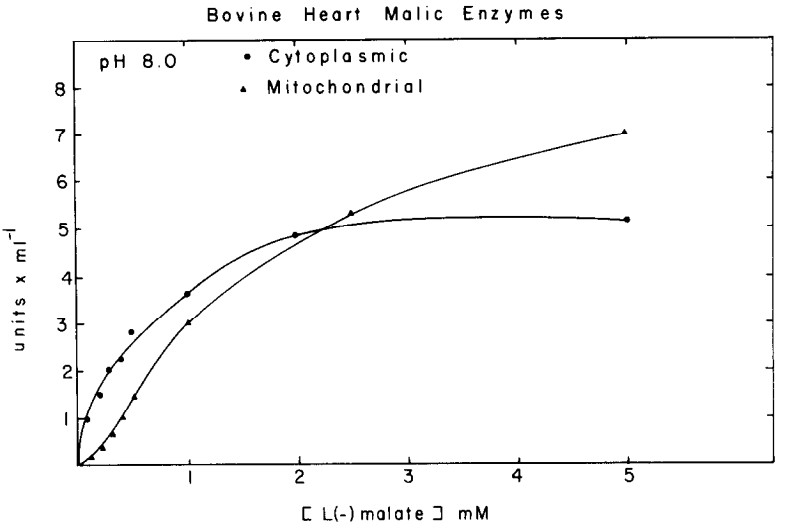

Figure 1. Effect of malate concentration on the oxidative decarboxylation of malate by the mitochondrial and cytoplasmic malic enzymes. Reaction rates were measured at 340 nm in a final volume of 2 ml in a reaction mixture containing: N-morpholinopropane sulfonate, pH 8.0, 100 mM; $MgCl_2$, 10 mM, $NADP^+$, 0.25 mM. After the addition of the indicated concentrations of L(-) malate, the reactions were started by the addition of 10 µliters of either cytoplasmic (● 0.58 mg ml^{-1}; specific activity 8.9 units mg^{-1}) or mitochondrial (▲ 0.75 mg ml^{-1}; specific activity 9.5 units mg^{-1}) malic enzyme. Activities are expressed as units per ml of the enzyme solution. Temperature: 25°.

As indicated in the figure, the cytoplasmic enzyme appears to follow classical enzyme kinetics (double reciprocal representations of these data do indeed yield linear plots). The mitochondrial isozyme, on the other hand, shows a distinct sigmoidicity at low malate concentrations. Calculations of the Michaelis constant for malate for the cytoplasmic isozyme give a value between 1×10^{-4} M and 3.5×10^{-4} M. No such calculations can be made in the case of the mitochondrial isozyme, due to the sigmoidal characteristics of the sub-

strate-velocity curve, which produces non-linear plots in the double reciprocal representation. It is obvious from the above figure that even if the maximal velocity of the mitochondrial malic enzyme obtained with the particular conditions used exceeds that measured with its cytoplasmic counterpart, at concentrations of malate below 2 mM the enzymic velocity of the cytoplasmic isozyme is far greater than that of the mitochondrial preparation.

All attempts to modify the cooperativity shown by the mitochondrial malic enzyme with respect to malate by using adenine nucleotides were not successful in changing the reaction rates at low malate concentrations. The addition of succinate, on the other hand, could modify significantly the kinetics of the reaction. This is indicated in Figure 2. The insert shows in greater detail the effects of succinate at malate concentrations below 1 mM. At these substrate concentrations there is a clear stimulation of the reaction rate when 10 mM succinate is added to the reaction cuvette.

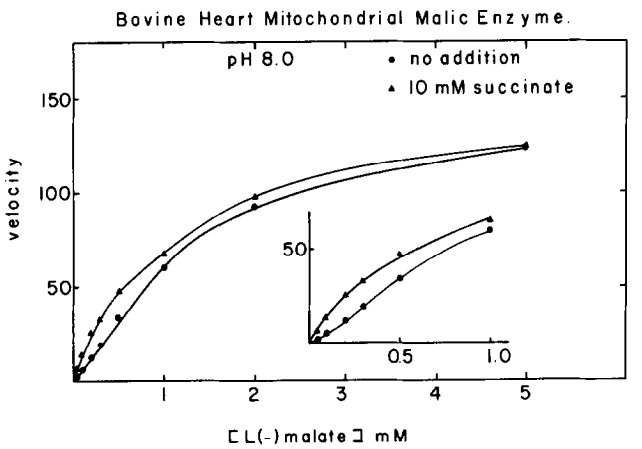

Figure 2. Effect of succinate on bovine heart mitochondrial malic enzyme. Reaction rates were measured as indicated in the legend to Figure 1, in the absence or presence of 10 mM succinate. Velocities are expressed as change in Absorbance at 340 nm per minute.

A better appreciation of the effects of succinate can be seen in Figure 3, where the ratios of the velocity in the presence and absence of succinate have been plotted as a function of the malate concentration. As indicated in the

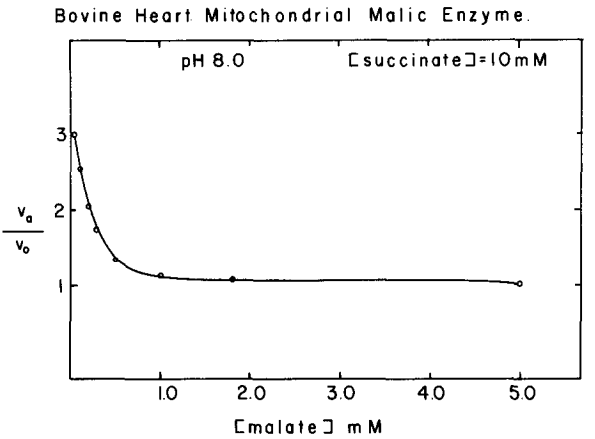

Figure 3. Activation of bovine heart mitochondrial malic enzyme by succinate at different concentrations of malate. The reaction rates in the presence (v_a) and absence (v_o) of 10 mM succinate were determined as indicated in Figure 1.

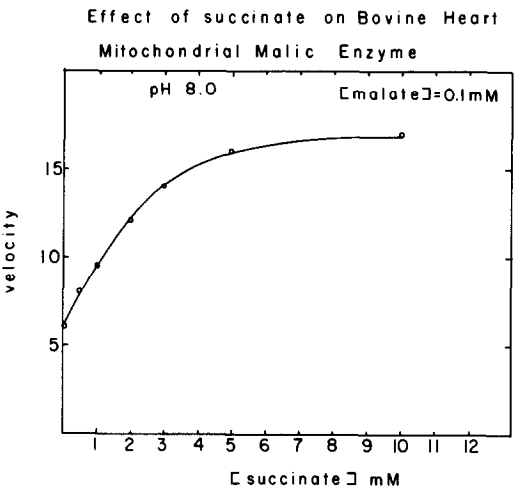

Figure 4. Effect of succinate on bovine heart mitochondrial malic enzyme. Reaction rates were determined as indicated in Figure 1, at a final concentration of malate of 0.1 mM, with the indicated concentrations of succinate. Velocities are expressed as change in Absorbance per minute at 340 nm.

figure, there is no apparent activation at malate concentrations exceeding 1 mM.

Figure 4 shows the dependence of the activation of the mitochondrial malic enzyme on the concentration of succinate, at a fixed concentration of malate (0.1 mM). The maximal amount of activation is observed at a 10 mM concentration of

succinate. Higher concentrations of succinate (not shown here) produce a small inhibition of the enzymic reaction, probably due to competition of succinate with malate for the active site.

The apparent activation constant for succinate, determined as indicated in Figure 5, is 3.7 mM. A similar value has been obtained at different malate concentrations and also at pH 7.5.

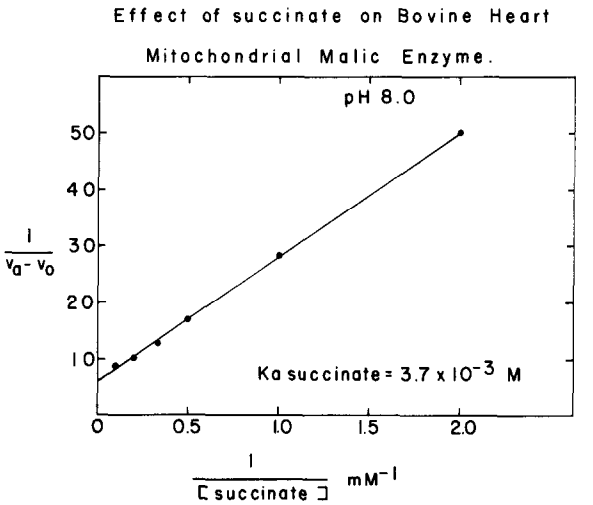

Figure 5. Determination of the activation constant of succinate in bovine heart mitochondrial malic enzyme. Reaction rates were determined as shown in Figure 1. v_a = rate in the presence of succinate; v_o = rate in the absence of succinate. Malate concentration = 0.1 mM.

Similar experiments to those indicated above, but using the cytoplasmic isozyme of malic enzyme, failed to demonstrate any effect, except a small amount of inhibition at concentrations of succinate exceeding 10 mM.

These observations further differentiate the two isozymes of heart malic enzyme and suggest that their metabolic roles may be quite different. While the cytoplasmic isozyme appears to be readily reversible, no such reversibility is seen with the mitochondrial enzyme in the absence of an effector (R. Frenkel, in preparation). These characteristics originally led Simpson and Estabrook (4) to suggest a possible role for the malic enzyme isozymes of adrenal cortex as mediators in an electron shuttle between cytosol and mitochondria. No such func

tion has been proven in the case of heart muscle, but the observations mentioned in this paper suggest that the activity of the mitochondrial malic enzyme may be regulated by the concentration of succinate as well as malate. Work is currently in progress with isolated heart mitochondria in an attempt to determine the possible function of malic enzyme in these organelles.

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