PURIFICATION AND PROPERTIES OF BEEF HEART MUSCLE "CYTOPLASMIC" MALIC DEHYDROGENASE*

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We have recently reported the occurrence of two malic dehydrogenases in minces of beef heart muscle which differ in sensitivity to inhibition by oxaloacetate (Siegel and Englard, 1960). A "mitochondrial" enzyme recognized by its strong capacity for inhibition by oxaloacetate, is extracted from fresh minces only when relatively drastic procedures are used for solubilization. These findings are in agreement with similar observations reported for other tissues (Delbrück, Zebe and Bücher, 1959; Delbrück, Schimassek, Bartsch and Bücher, 1959). The enzyme which behaved kinetically like "mitochondrial" malic dehydrogenase was purified from extracts of acetone-dried powders of whole beef heart by following the essentials of the procedure originally described by Straub (1942) (Siegel and Englard, 1960). The molecular properties of the malic dehydrogenase thus obtained, differed greatly from those reported by Davies and Kun (1957) for the enzyme isolated and purified from acetone-dried powders of ox heart mitochondria, and more closely resembled the enzyme prepared by Wolfe and Neilands (1956) from whole pig heart acetone powders.

In view of the marked differences in molecular properties of malic dehydrogenase isolated from acetone-dried powders of ox heart mitochondria and the "mitochondrial"

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enzyme obtained from acetone-dried powders of thoroughly washed minces of whole beef heart, it was of interest to investigate the physico-chemical properties of the beef heart "cytoplasmic" enzyme. In addition, such a study is a prerequisite for the proposed structural studies of the various enzymes under consideration. The present communication, therefore, reports the preparation and some kinetic properties of purified "cytoplasmic" malic dehydrogenase from beef heart.

Fresh beef hearts were defatted, diced and passed through a mechanical grinder. The mince was suspended in 5 volumes of cold 0.25M sucrose + 0.01M triethanolamine pH 7.6 and stirred mechanically for 15 minutes. The suspension was decanted through several layers of cheese cloth and the filtrate fractionated between 40 and 82 percent saturation with solid ammonium sulfate. During this and subsequent ammonium sulfate fractionations the pH was maintained between 7.2 and 7.4 by the addition of dilute ammonium hydroxide. The 40 to 82 percent residue was dissolved in 0.05M potassium phosphate + 0.001M ethylenediamine tetraacetate (EDTA) pH 7.4 and without prior dialysis brought to 35 percent saturation with ammonium sulfate. After centrifugation, the clear supernatant was rapidly brought to 60° by immersion in a boiling water bath and the solution was maintained between 62 to 64° for 10 minutes. After cooling, the bulky precipitate was removed by centrifugation and the supernatant solution raised to 45 percent saturation with ammonium sulfate. The precipitate was removed by centrifugation and ammonium sulfate was added to the supernatant solution to 72 percent saturation. The 45 to 72 percent ammonium sulfate fraction was dialyzed against 0.035M potassium phosphate + 0.0035M EDTA pH 6.4 and subsequently diluted seven fold with water. This solution was passed through a DEAE-cellulose column equilibrated with 0.005M potassium phosphate + 0.0005M EDTA pH 6.4. The last traces of "mitochondrial" enzyme still present at this stage of purification were eliminated by this procedure since this enzyme was not retained by the DEAE-cellulose. A similar unretarded behavior of rat liver "mitochondrial" malic dehydrogenase on DEAE-cellulose has recently been reported (Thorne, 1960). The "cytoplasmic" malic dehydro-

genase was eluted with 0.02M potassium phosphate + 0.002M EDTA pH 6.4 at a flow rate of 10 to 12 ml. per hour. Fractions of an increased specific activity of 5 fold or greater were pooled and the enzyme concentrated by precipitation with ammonium sulfate. The enzyme was further purified by starch block electrophoresis in 0.038 ionic strength barbital buffer at pH 8.5. The enzyme thus obtained moved as a single electrophoretic component toward the anode at pH 7.1 in 0.05M potassium phosphate + 0.001M EDTA.

The purified "cytoplasmic" malic dehydrogenase had a higher specific activity when assayed at pH 7.5 in 0.025M glycylglycine (Ochoa, 1955) than the purified "mitochondrial" enzyme isolated from acetone-dried powders of whole beef heart muscle (Table I). However, unlike the "mitochondrial" enzyme which showed increased activity in 0.05M potassium phosphate* (with or without glycylglycine), the "cytoplasmic" enzyme is unaffected in the presence of added phosphate.

At pH 6.7 (0.5M potassium phosphate), concentrations of oxaloacetate as high as 1.9×10^{-3}M showed no inhibitory effect on the "cytoplasmic" enzyme. This is in marked contrast with the characteristic inhibition of "mitochondrial" malic dehydrogenase, evident at an oxaloacetate concentration of 2.7×10^{-4}M with the enzyme purified from acetone powders of whole beef heart (Siegel and Englard, 1960). In addition, unlike the beef heart "mitochondrial" enzyme which exhibits an anamolous acceleration of the rate of DPN^+ reduction at high concentrations of L-malate (Siegel and Englard, 1960) the beef heart "cytoplasmic" malic dehydrogenase appears to be sensitive to levels of L-malate exceeding 1.6×10^{-2}M when assayed in 0.1M tris (hydroxymethyl) aminomethane at pH 8.4.

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* The malic dehydrogenase obtained from whole pig heart acetone powders behaves in a similar manner and this effect has also been obtained with sulfate, arsenate, maleate, EDTA, etc. and appears to be due to the increased ionic strength of the assay medium. The enzyme obtained directly from acetone-dried powders of beef heart mitochondria also shows increased activity when assayed in glycylglycine in the presence of potassium phosphate. It thus appears that the stimulatory effect of added salts is characteristic for the enzymes which behave kinetically like "mitochondrial" malic dehydrogenase.

Table 1

Comparison of Purified Beef Heart "Cytoplasmic" Malic Dehydrogenase with the Purified "Mitochondrial" Malic Dehydrogenase Isolated from Acetone-Dried Powders of Whole Beef Heart

Conditions of Assays	Turnover Number* "Cytoplasmic" enzyme	Turnover Number* "Mitochondrial" enzyme**
DPNH OXIDATION WITH OXALOACETATE:		
a) in 0.025M glycylglycine pH 7.5(1)	817	585
b) in 0.025M glycylglycine + 0.05M potassium phosphate pH 7.5(1)	817	1493
c) in 0.05M triethanolamine pH 7.6(2)	701	1088
d) in 0.5M potassium phosphate pH 6.7(3)	487	738
DPNH OXIDATION WITH MESOXALATE:		
a) in 0.5M potassium phosphate pH 6.7(4)	13	63
DPN^+ REDUCTION WITH L-MALATE:		
a) in 0.09M glycine-NaOH pH 9.9(5)	94	527
b) in 0.1M tris (hydroxymethyl) aminomethane pH 8.4(6)	87	115
DPN^+ REDUCTION WITH MESO-TARTRATE:		
a) in 0.1M glycine-NaOH pH 9.9(7)	0.32	2.33
DPN^+ REDUCTION WITH L-TARTRATE:		
a) in 0.1M glycine-NaOH pH 9.9(8)	0.44	4.27

* Expressed as µmoles of either DPNH oxidized or DPN^+ reduced per minute per mg of protein, as calculated from the initial reaction rates recorded with a Cary Spectrophotometer.

** cf. Siegel and Englard (1960).

In addition, each of the above reaction mixtures contained:

(1) 0.76 µmoles of oxaloacetate and 0.26 mg of DPNH in a total volume of 3.02 ml.

(2) 0.38 µmoles of oxaloacetate and 0.39 mg of DPNH in a total volume of 3.02 ml.

(3) 6.4 µmoles of EDTA, 0.76 µmoles of oxaloacetate and 0.31 mg of DPNH for the "cytoplasmic" enzyme assay and 0.26 mg of DPNH for the "mitochondrial" enzyme assay, in a total volume of 3.02 ml.

(4) 6.4 µmoles of EDTA, 25.0 µmoles of mesoxalate and 0.26 mg of DPNH in a total volume of 3.01 ml.

(5) 300 µmoles of sodium-L-malate and 7.36 µmoles of DPN^+ in a total volume of 3.02 or 3.05 ml. The concentration of L-malate used was somewhat inhibitory for the "cytoplasmic" enzyme. Under the same conditions in the presence of only 50 µmoles of sodium-L-malate the "cytoplasmic" enzyme yielded a turnover number of 118.

(6) 250 µmoles of sodium-L-malate and 8.61 µmoles of DPN^+ for the "cytoplasmic" enzyme assay and 11.77 µmoles of DPN^+ for the "mitochondrial" enzyme assay, in a total volume of 16.0 or 16.1 ml.

(7) 500 µmoles of sodium-meso-tartrate and 7.36 µmoles of DPN^+ in a total volume of 3.02 or 3.1 ml.

(8) 500 µmoles of sodium-L-tartrate and 8.37 µmoles of DPN^+ in a total volume of 3.02 or 3.1 ml.

Table I also shows other kinetic differences between the purified beef heart "cytoplasmic" and "mitochondrial" enzymes as evidenced by the different relative rates for the oxidation and reduction of DPNH and DPN^+, respectively, in the presence of several substrates as assayed in various buffers at different pH values.

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