MALIC DEHYDROGENASE FROM ACETONE-DRIED POWDERS OF WHOLE BEEF HEART MUSCLE*

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Wolfe and Neilands (1956), introducing a zinc-ethanol step into the method of purification for malic dehydrogenase first reported by Straub (1942), obtained an homogeneous preparation of the enzyme from acetone-dried powders of whole pig heart, and reported it to have a molecular weight of 40,000 ± 5,000. Applying the essential features of the Straub procedure (1942) to acetone-dried powders of ox heart mitochondria, Davies and Kun (1957) prepared a purified malic dehydrogenase with a reported molecular weight of 15,000-20,000 and which also differed markedly in other physical constants from the enzyme isolated by Wolfe and Neilands (1956) from pig heart. Although the reported physical differences may represent actual species characteristics, the possibility that malic dehydrogenase occurs in several different molecular forms was considered by Davies and Kun (1957) and is inherent in the prior observation of Wolfe (1955) that the pig heart enzyme was separable into two active fractions by chromatography on carboxymethylcellulose. Support for the concept of multiple molecular varieties of malic dehydrogenase may also be inferred from recent reports (Christie and Judah, 1954; Delbrück, Zebe and Bücher, 1959; Delbrück, Schimassek, Bartsch and Bücher, 1959; Wieland et al, 1959) of "cytoplasmic" and "mitochondrial" preparations differing in electrophoretic mobility and kinetic properties.

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A characteristic inhibition of "mitochondrial" malic dehydrogenase by high concentrations of oxaloacetate has been described (Delbrück, Zebe and Bücher, 1959; Delbrück, Schimmassek, Bartsch and Bücher, 1959) and demonstrated to apply to the enzyme obtained from acetone-dried ox heart mitochondria (Davies and Kun, 1957) or acetone-dried powders of whole pig heart (Pfleiderer and Hohnholz, 1959). The latter preparation was made using conditions more closely adhering to those of the original procedure of Straub (1942). The marked differences between reported physical properties of these preparations, both presumably mitochondrial in origin, may therefore actually reflect species differences between the enzymes per se. In view of the fact that a "mitochondrial" enzyme was prepared directly from whole pig heart, it was thought feasible to isolate the corresponding lower molecular weight enzyme from whole beef heart without first engaging in tedious separation of mitochondria. If such a procedure were successful it should provide large quantities of "mitochondrial" malic dehydrogenase of beef heart, a prerequisite for proposed structural studies of that enzyme. In turn, the latter program is part of our larger aim to determine the pertinent structural features of representative enzymes catalyzing stereospecific hydrogen transfer reactions. The present communication, therefore, reports the preparation and properties of a purified "mitochondrial" malic dehydrogenase from beef heart muscle.

First, in agreement with observations reported for other tissues (Delbrück, Zebe and Bücher, 1959; Delbrück, Schimassek, Bartsch and Bücher, 1959), we found that beef heart muscle contains two forms of malic dehydrogenase which differ in sensitivity to inhibition by oxaloacetate (see Table I). From the ratios of enzymatic activities measured at low and high oxaloacetate concentrations, it may be seen in this table that "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.

This enzyme, unlike "cytoplasmic" malic dehydrogenase, is relatively stable to treatment with organic solvents, and can be extracted from acetone-dried powders of thoroughly washed minces of whole beef heart. For purification, therefore, extracts of such powders were treated

Table I

Differential Extraction of Beef Heart Malic Dehydrogenase

Conditions of sequential extractions*	Number of extractions using indicated conditions	Total units of malic dehydrogenase in supernate**	Specific activity***	Ratio of low:high oxaloacetate assay
Mechanical stirring with 0.25 M sucrose + 0.01 M triethanolamine pH 7.3	1	431	9.5	1.12
	2	45	-	1.15
	3	14	-	1.22
Homogenization in teflon homogenizer with 0.25 M sucrose + 0.01 M triethanolamine pH 7.3	1	55	-	2.25
	2	28	-	2.50
	3	22	-	2.62
Homogenization in glass homogenizer with 0.1 M $K-PO_4$ pH 7.4	1	211	15.8	3.17
	2	46	-	2.32
Homogenization in high speed Lourdes homogenizer with 0.1 M $K-PO_4$ pH 7.4	1	115	35.8	2.47
	2	46	-	2.44
	3	19	-	2.40

The conditions for assaying malic dehydrogenase activity were the same as those described by Delbrück, Zebe and Bücher (1959). The low oxaloacetate assays were run at a substrate concentration of 1.2×10^{-4} M and the high oxaloacetate assays at a concentration of 1.2×10^{-3} M. Initial rates of DPNH oxidation were recorded with a Cary Spectrophotometer at 360 mμ.

* After each extraction the supernatant fraction was collected by centrifugation between 78,000 and 105,000 x g for 20 minutes in a preparative Spinco ultracentrifuge. The residue was then quantitatively recovered and subjected to a subsequent indicated extraction procedure.

** Expressed as μmoles of DPNH oxidized per minute per gram wet weight of freshly minced tissue as assayed at the low oxaloacetate concentration. The recovery of malic dehydrogenase activity for the combined fractions was equivalent to that of an independent experiment in which the tissue was exhaustively extracted by the single procedure of blending in a Lourdes high speed homogenizer with 0.1 M $K-PO_4$ pH 7.4.

*** Expressed as μmoles of DPNH oxidized per minute per mg of protein as assayed at the low oxaloacetate concentration.

by the method of Straub (1942) as modified by Ochoa (1955). The final treatment with ammonium sulfate, however, was carried out in a stepwise manner between 60 and 75 per cent saturation, and a number of active fractions obtained. Those with the highest specific activities were equilibrated by dialysis with 30 per cent ammonium sulfate solution also 0.2 M with respect to potassium phosphate buffer, pH 7.4. These fractions were next heated at 60°

for 2 minutes and centrifuged. The controlled heating step resulted in removal of considerable non-specific protein. Enzyme preparations obtained in this manner had considerably higher specific activities than malic dehydrogenase isolated from acetone-dried powders of ox heart mitochondria or whole pig heart (Table II).

Table II

Comparison of Whole Beef Heart Acetone Powder Malic Dehydrogenase With Other Reported Preparations

Properties	Enzyme from acetone-dried powders of whole beef heart	Enzyme from acetone-dried powders of ox heart mitochondria*	Enzyme from acetone-dried powders of whole pig heart**
D°_{20}	$6.45 \times 10^{-7} cm^2 sec^{-1}$	$13.5 \times 10^{-7} cm^2 sec^{-1}$	$8.47 \times 10^{-7} cm^2 sec^{-1}$
S°_{20}	$4.3 \times 10^{-13} sec$	$2.1 \times 10^{-13} sec$	$3.6 \times 10^{-13} sec$
Molecular weight	62,000	15,000-20,000	40,000 + 5000***
Isoelectric point	5.5 - 5.6	> 6.9	6.1 - 6.4
Turnover numbers****			
a) DPNH oxidation at pH 7.4 in phosphate buffer	1493	750	950
b) DPN^+ reduction at pH 10.0 in glycine-NaOH buffer	527	306	243

* Data from Davies and Kun (1957).

** Data from Wolfe and Neilands (1956).

*** Purification of malic dehydrogenase from acetone-dried powders of whole pig heart by the method of Straub (1942) as modified slightly by Ochoa (1955), yielded a homogeneous preparation with a molecular weight of approximately 56,000 (Englard and Franzblau, unpublished data).

**** Expressed as μmoles of either DPNH oxidized or DPN^+ reduced per minute per mg of protein. The assay conditions for the comparative turnover numbers, although similar, were not identical.

The characteristic inhibition of "mitochondrial" malic dehydrogenase by high concentrations of oxaloacetate was evident with the present preparation at a substrate level as low as 2.7×10^{-4} M when assayed in 0.5 M $K\text{-}PO_4$ at pH 6.7. The kinetic data with malate obtained with the present enzyme, represented graphically in a double reciprocal plot, 1/v against 1/S, (Lineweaver and Burk, 1934) yield a slope which increases with in-

creasing substrate concentration (assay buffer, 0.1 M glycine-NaOH, pH 10.0). This anomalous behavior was observed by Wolfe and Nielands (1956) and Davies and Kun (1957) with their respective preparations, and is unlike the behavior of the beef heart "cytoplasmic" malic dehydrogenase. The latter enzyme is inhibited by higher concentrations of malate (Englard et al., 1960).

Electrophoretic analysis at various pH values ranging from 4.5 to 6.9 revealed only a single component, and measurements of mobilities permitted assignment of an isoelectric point at pH 5.5-5.6. The purified enzyme sedimented in the ultracentrifuge as a single component with an S°_{20} value of 4.3×10^{-13} sec, and exhibited a D°_{20} value of 6.45×10^{-7} $cm^2 sec^{-1}$. Using these data and assuming a partial specific volume of 0.74 $ml\text{-}gm^{-1}$, a molecular weight of approximately 62,000 was calculated.

Although the enzyme purified from extracts of acetone-dried powders of beef heart behaves kinetically like "mitochondrial" malic dehydrogenase, it differs greatly in molecular properties from enzyme isolated from acetone-dried powders of ox heart mitochondria. It more closely resembles malic dehydrogenase prepared from acetone-dried powders of whole pig heart (see Table II).

References

Christie, G.S., and Judah, J.D., Proc. Roy. Soc. B, 141, 420 (1954).

Davies, D.D., and Kun, E., Biochem. J., 66, 307 (1957).

Delbrück, A., Zebe, E., and Bücher, T., Biochem. Z., 331, 273 (1959).

Delbrück, A., Schimassek, H., Bartsch, K., and Bücher, T., Biochem. Z., 331, 297 (1959).

Englard, S., Siegel, L., and Breiger, H.H., Biochem. and Biophys. Research Communications, 3, 323 (1960).

Lineweaver, H., and Burk, D., J. Am. Chem. Soc., 56, 658 (1934).

Ochoa, S., in Colowick, S.P., and Kaplan, N.O., Methods in Enzymology, 1, 735, Academic Press, Inc., New York (1955).

Pfleiderer, G., and Hohnholz, E., Biochem. Z., 331, 245 (1959).

Straub, F.B., Z. Physiol. Chem., 275, 63 (1942).

Wieland, T., Pfleiderer, G., Haupt, I., and Wörner, W., Biochem. Z., 332, 1 (1959).

Wolfe, R.G., Ph.D. Thesis: University of California (1955).

Wolfe, R.G., and Neilands, J.B., J. Biol. Chem., 221, 61 (1956).