MULTIPLE FORMS OF MITOCHONDRIAL MALATE DEHYDROGENASES*

G. B. Kitto,** P. M. Wassarman,† J. Michjeda‡ and N. O. Kaplan

From the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts (Publication No. 395)

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A previous report from this laboratory indicated that, in addition to the mitochondrial and supernatant malate dehydrogenases (MDH's), several bands possessing catalytic activity could be observed during starch-gel electrophoresis of mitochondrial MDH (1). The catalytic properties of the separate bands eluted from the gel appeared to be quite similar.

In the present investigation chromatography on carboxymethyl cellulose (CMC), pH 6.5, was used to obtain fractions containing predominantly single bands as demonstrated by starch-gel electrophoresis. The catalytic properties of the individual bands were compared by means of coenzyme analogues and found to be nearly identical. Resistance to heat denaturation, which was used to differentiate lactic dehydrogenases (LDH's) and their hybrids (2), failed to show any significant differences among the bands.

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** Durkee Graduate Fellow of the Glidden Company.
† Predoctoral trainee, National Institute of General Medical Science training grant No. 5T1 GM 212-06.
‡ Present address: Department of Animal Physiology, Poznan University, Poznan, Poland.

The presence of multiple bands could be caused by formation of different proteins by duplicate genes or by changes in conformation of the enzyme. Charge differences caused by differential binding of cofactor or varying degrees of oxidation of sulfhydryl groups, which has been suggested as an explanation for the microheterogeneity of LDH's (3), appear to be unlikely for mitochondrial MDH in view of the following experiments. When samples were run on starch-gel containing 5×10^{-3} M β-mercaptoethanol in both the gel and buffer or preincubated for 15 min. in 0.1 M β-mercaptoethanol before electrophoresis in the above system, the pattern of enzyme activity was identical to that in the absence of β-mercaptoethanol.

The individual bands were reacted by the Ouchterlony double diffusion technique (4) with a rabbit antibody directed against crystalline chicken heart mitochondrial MDH containing all of the bands. A single sharp fused precipitin line was observed for each band. To test the possibility that some of the bands might result from the hybridization of mitochondrial MDH with the supernatant enzyme, the separate bands were cross reacted in the Ouchterlony system with an antibody directed against ostrich heart supernatant MDH that was known to cross react strongly with the chicken supernatant enzyme. No cross reaction between the mitochondrial enzyme forms and the antibody to the supernatant enzyme could be observed even at high antibody or antigen concentrations. Further confirmation of this non-identity between supernatant and mitochondrial forms was obtained by demonstrating that a hybrid prepared from the pig soluble and mitochondrial enzymes had electrophoretic properties unlike any bands of either parent enzyme.*

Several experiments were performed to determine the time

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course for inhibition of chicken mitochondrial MDH by hydroxymercuribenzoate (HMB). Specific bands were isolated by CMC column chromatography and subjected to HMB titration. The results indicate (Fig. 1) that the bands exhibitng the most anodic character on starch-gel electrophoresis are the most sensitive to inhibition by HMB. The more cathodic bands exhibit an increasing resistance to inactivation by HMB.

Chicken and pig heart mitochondrial MDH were iodinated by

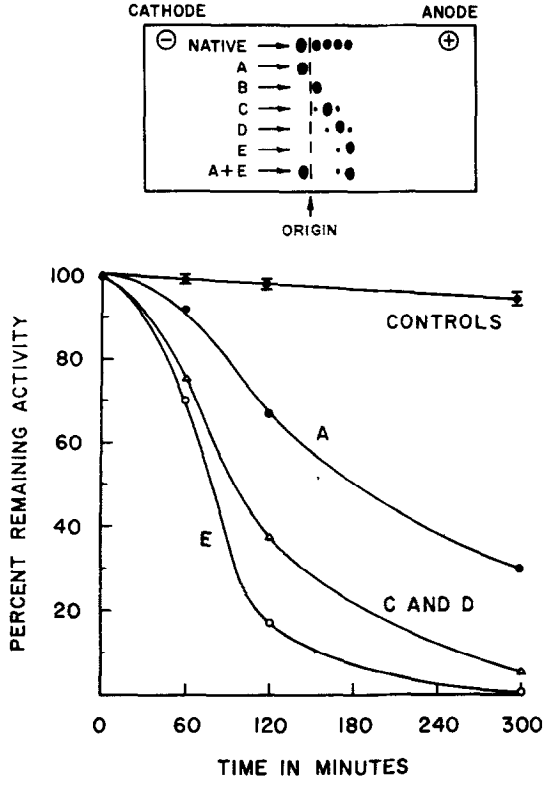

Fig. 1. Top. A tracing of a starch gel electrophoresis of chicken heart mitochondrial MDH fractionated by CMC chromatography. Electrophoresis was performed at pH 7.0 in phosphate citrate buffer for 16 hrs at 10 volts per cm. Samples A-E were successive fractions eluted from the CMC column; fraction E was the first to be eluted. Bottom. Time course of inhibition by HMB of separate bands of chicken heart mitochondrial MDH. HMB concentration was 10^{-5} M. Controls contained no mercurial.

the method of McFarlane (5) under conditions reported as being specific for the substitution of iodine in the 3,5-positions of tyrosine residues. The effect of iodination on the catalytic activity of both the chicken and pig enzymes is shown in Fig. 2. It is apparent that both pig and chicken mitochondrial MDH's may be modified at a low level of iodination without loss of enzymatic activity. Contrary to the inhibitory action of HMB, the cathodic

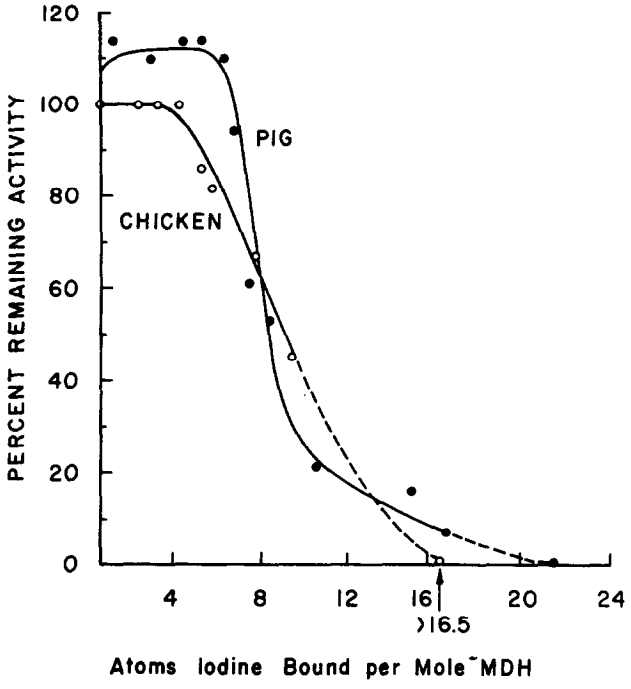

Fig. 2. Effect of iodination on the enzymatic activity of chicken and pig heart mitochondrial MDH. Iodinations were carried out in 0.1 M borate buffer, pH 9.2, at a protein concentration of 1.0 mg/ml.

bands of mitochondrial MDH were most sensitive to inhibition by iodine. At the level of iodination initiating a decrease in catalytic activity, there was an accompanying decrease in the amount of the most cathodic enzyme band; in addition, there was a concomitant increase in the intensity of some of the more anodic bands,

as with chicken mitochondrial MDH, or the formation of new anodic bands, as with the corresponding pig enzyme (Figs. 3 and 4).

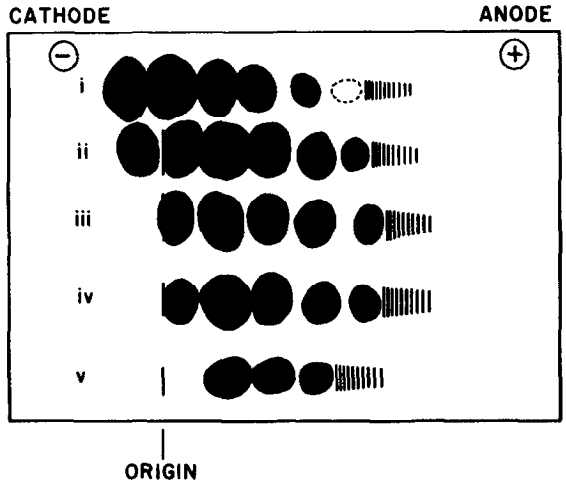

Fig. 3. Effect of iodination on the multiple forms of chicken heart mitochondrial MDH. Photograph of a starch-gel electrophoresis performed at pH 7.0 in phosphate-citrate buffer for 16 hrs. at 10 volts per cm. (i) native control, (ii) 2.0, (iii) 4.0, (iv) 7.5, and (v) 9.3 atoms of iodine bound per mole MDH.

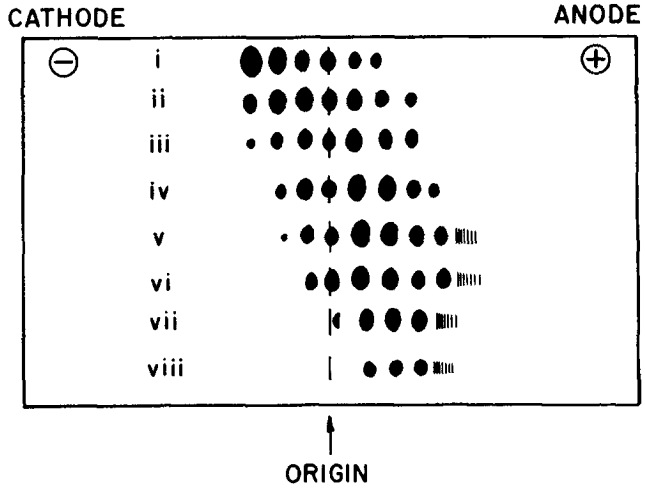

Fig. 4. Effect of iodination on the multiple forms of pig heart mitochondrial MDH. Tracing of a starch-gel electrophoresis performed at pH 7.0 in phosphate-citrate buffer for 16 hrs. at 10 volts per cm. (i) native control, (ii) 1.2, (iii) 2.6, (iv) 3.8, (v) 5.0, (vi) 6.5, (vii) 8.2, and (viii) 12.8 atoms of iodine bound per mole MDH.

When duplicate slices of starch-gel were stained for protein with amido black, mobility changes of the protein bands paralleled changes in the pattern of enzymatic activity.

Samples of iodinated chicken heart mitochondrial MDH, which had lost varying proportions of their enzymatic activity, were reacted with a rabbit antibody prepared against the native enzyme, by the quantitative semimicro-complement fixation technique (6). An exact correspondence between loss of activity and complement fixing ability was found. It was apparent that the iodinated enzyme that had lost catalytic activity had also completely lost its immunological reactivity at the antibody concentration employed; the remaining active enzyme was able to fix complement in the normal manner.

These results, together with those of Thorne et al.(1), who observed modifications in the gel electrophoretic patterns of mitochondrial MDH's treated with urea and methyl iodide, indicate that it is possible to modify selectively the nature of the multiple forms of mitochondrial MDH's. Unlike hybrids of LDH, which are a combination of dissimilar peptide chains (7), mitochondrial MDH's, as indicated by preliminary peptide analyses, are composed of nearly identical subunits.* Work directed toward the isolation of separate bands of crystalline chicken heart mitochondrial MDH in sufficient quantities for amino acid analysis and peptide mapping is in progress and should provide considerably more insight into the nature of the observed microheterogeneity.

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