PRELIMINARY NOTE

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Nature of the isozymes of rat liver mitochondrial malate dehydrogenase

Rat liver mitochondrial malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37) has a molecular weight of 66 300, and is composed of two subunits of equal molecular weight[1]. The enzyme, which can be purified by the method of SOPHIANOPOLOUS AND VESTLING[2], can be separated into three major and two minor catalytically active components as shown by starch-gel electrophoresis. Isozymes of mitochondrial malate dehydrogenase, which have been reported for a number of species[3,4] have been designated by KITTO *et al.*[5] as A, B, C, D and E, beginning with the most cationic isozyme. Rat liver mitochondrial malate dehydrogenase preparations are composed predominantly of isozymes A, B and C, with very small amounts of D and E.

KITTO, WASSARMAN AND KAPLAN[6] have carried out optical rotatory dispersion, reversible denaturation and immunological studies on separated isozymes of chicken heart mitochondrial malate dehydrogenase. From these studies the authors have concluded that the chicken heart mitochondrial malate dehydrogenase isozymes are conformational isomers of the same polypeptide chain(s).

Recently SCHECHTER AND EPSTEIN[7] have reported reversible dissociation studies on partially resolved chicken heart mitochondrial malate dehydrogenase isozymes. These studies, carried out in 7.6 M guanidine·HCl failed to substantiate the "conformer" hypothesis of KITTO *et al.*[6]. While KITTO *et al.*[6] reported that reversible dissociation of isozyme E at pH 2.0 resulted in the production of the entire distribution of isozymes, SCHECHTER AND EPSTEIN[7] have reported that the reversible dissociation of a mixture of isozymes C, D and E in guanidine·HCl resulted in no change in the electrophoretic distribution of these isozymes.

The present communication deals with experiments performed in our laboratory which appear to offer a different view of the mitochondrial malate dehydrogenase isozyme system. The principal rat liver mitochondrial malate dehydrogenase isozymes have been partially resolved using a CM-Sephadex column equilibrated with 0.02 M sodium citrate (pH 6.0). The enzyme was eluted with the aid of a pH gradient established by the presence of 0.02 M sodium citrate (pH 6.0) in the mixing flask, and 0.02 M sodium citrate (pH 7.0) in the reservoir. The chromatographic profile from this experiment indicated three distinct overlapping peaks. Selected pooling of fractions from each of the three peaks resulted in partial resolution of isozymes A, B and C.

The separated isozymes obtained from the column were subjected to reversible dissociation at pH 2 in 0.1 M 2-mercaptoethanol and renaturation by dilution with 0.5 M sodium citrate (pH 7.0) containing 0.1 M 2-mercaptoethanol. This reversible dissociation technique is a modification of the method of CHILSON, KITTO AND KAPLAN[8].

Volumes containing 0.5 ml of each isozyme solution were titrated to pH 2.0 by the addition of 0.5 ml of 0.03 M HCl, 0.1 M 2-mercaptoethanol in an ice bath, and allowed to remain at pH 2.0 for 15 min. At the end of this interval, the solutions were neutralized by dilution with 10 ml of 0.5 M sodium citrate, 0.1 M 2-mercaptoethanol

(pH 7.0) and allowed to stand at 25° for 2 h. The recoveries of enzymatic activities were 77, 53 and 83% for isozymes A, B and C, respectively. The treated isozyme samples were concentrated by ultrafiltration and ammonium sulfate precipitation, dialyzed and subjected to electrophoretic analysis. The appropriate controls, subjected to each step of the procedure except acidification and neutralization, were diluted to the same enzyme concentration and matched with each treated isozyme sample. The electrophoretograms are shown in Fig. 1. In this photograph it can be seen that small amounts of contaminating isozymes were present in all the original samples. The

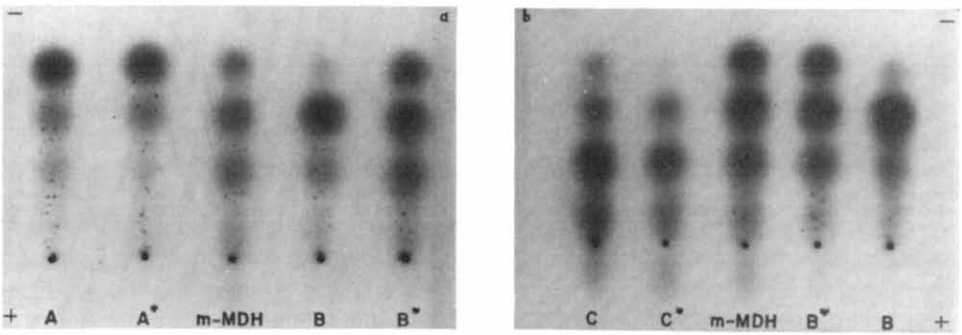

Fig. 1. a. Photograph of a starch-gel electrophoretogram of isozyme A, reversibly dissociated A (A*), purified rat liver mitochondrial malate dehydrogenase (m-MDH), isozyme B and reversibly dissociated B (B*). b. Photograph of a starch-gel electrophoretogram of isozyme C, reversibly dissociated C (C*), purified rat liver mitochondrial malate dehydrogenase (m-MDH), reversibly dissociated B (B*) and isozyme B.

reversibly dissociated samples are indicated by the letter designation for the isozyme followed by an asterisk. It can be seen that while isozymes A and C show no differences in their electrophoretic distribution following reversible dissociation, isozyme B gave isozymes A, B and C on reversible dissociation. The observed electrophoretic patterns for the reversibly dissociated A and C isozymes appear to rule out the possibility of selective inactivation of isozyme B, as both of these fractions (A and C) are seen to contain the same relative amounts of B both before and after exposure to pH 2.0 and renaturation.

From these studies, together with information about the molecular weights of the subunits of rat liver mitochondrial malate dehydrogenase[1], it has been concluded that isozyme A is a dimer composed of two X-type subunits, B is a hybrid dimer composed of one X-type subunit and one Y-type subunit and isozyme C is a dimer composed of two Y-type subunits.

The existence of isozymes D and E cannot be explained by the proposed hypothesis. However, the relative amounts of these two isozymes appear to increase during purification of the enzyme. These isozymes have even become the major components after prolonged exposure of the enzyme to particular lots of Amberlite IRF-97 (formerly XE-64, Rohm and Haas Co.). Preliminary studies of a mixture of mitochondrial malate dehydrogenase isozymes which contained isozymes D and E as major components indicate that such mixtures are unchanged in their electrophoretic behavior following reversible acid dissociation, and thus probably are not "conformers" of X- and Y-type subunits.

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