CHLOROPLAST-MALIC DEHYDROGENASE: A NEW MALIC DEHYDROGENASE ISOZYME FROM SPINACH

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Malic dehydrogenase (MDH) isozymes are well known in plant tissues. Observations on the intracellular localization of dehydrogenases (Price and Thimann, 1954) has prompted detailed studies of malic dehydrogenase isozymes. Because malic dehydrogenase activity has been reported in the chloroplasts of higher plants (Pierpoint, 1963), we hypothesized that chloroplasts would have a unique malic dehydrogenase protein. Schweiger, Master, and Werz (1967) reported that the main two MDH isozymes in *Acetabularia mediterranea* were associated with chloroplasts. In this report, evidence is presented for a new malic dehydrogenase isozyme which we have designated as chloroplast-malic dehydrogenase.

Methods: Chloroplasts, mitochondria, and soluble supernatant fractions were prepared from spinach leaves similarly to our earlier report (Mukerji and Ting, 1968). Fresh leaves were homogenized in a Waring Blendor at low speed with a medium containing 0.35 M sodium chloride in 0.05 M tris buffer, *p*H 8.0. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 200 g for 1 minute; the resulting pellet containing debris was discarded. Chloroplasts were isolated by centrifugation at 1,000 g for 10 minutes. A mitochondrial pellet was isolated from the supernatant by centrifugation at 10,000 g for 30 minutes. The resulting supernatant was designated as the soluble fraction. Both chloroplasts and mitochondria were

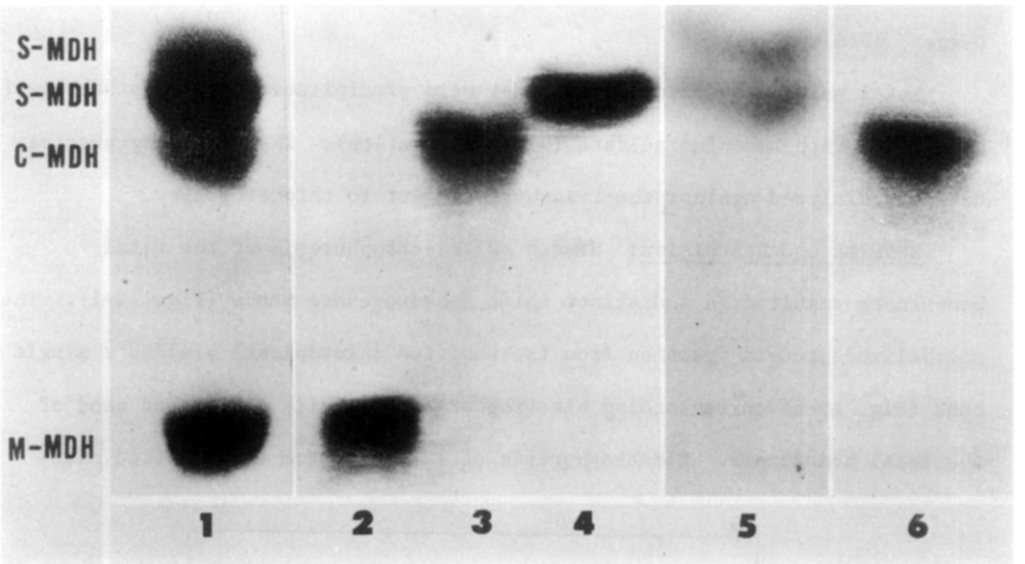

Fig. 1. Starch gel electrophoresis of malic dehydrogenase proteins from spinach leaves. (1) Partially purified homogenate from spinach leaf extract showing four distinct bands. (2) First peak from DEAE-cellulose column (refer to Fig. 2). (3) Second peak from DEAE-cellulose column. (4) Third peak from DEAE-cellulose column. (5) Shoulder associated with third peak of DEAE-cellulose column. (6) Band associated with purified chloroplasts. The latter corresponds exactly with the second electrophoretic band and the second peak from the DEAE-cellulose column. Electrophoretic mobilities were established by running all fractions on the same gel.

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purified by layering on a sucrose density gradient from 25 to 60% and centrifuging at 1,000 g for 45 minutes in a swing-out head. Electron micrographs indicated chloroplasts purified by this method were devoid of mitochondria and bacteria, but were associated with chloroplast membrane fragments. Enzymes associated with the purified organelles were solubilized by sonic irradiation. Isozymes were separated and purified by anion exchange column chromatography on a 1.5 x 15 cm DEAE-cellulose column. Elution was

with a phosphate gradient (*p*H 7.0) from 0.02 to 0.2 M as previously described (Ting, 1968). Starch gel electrophoresis was conducted according to the method of Fine and Costello (1968) as previously reported (Ting, Sherman and Dugger, 1966).

Total malic dehydrogenase isozymes were precipitated from a spinach leaf homogenate with ammonium sulfate (0-0.9 saturation). The resulting precipitate was dialyzed against the tris buffer prior to chromatography.

Results and Discussion: Starch gel electrophoresis of the total homogenate resulted in 4 distinct malic dehydrogenase bands (Fig. 1.-1). The solubilized protein fraction from the purified chloroplasts yielded a single band (Fig. 1.-6) corresponding electrophoretically with the second band of the total homogenate. Electrophoresis of extracts from the purified mito-

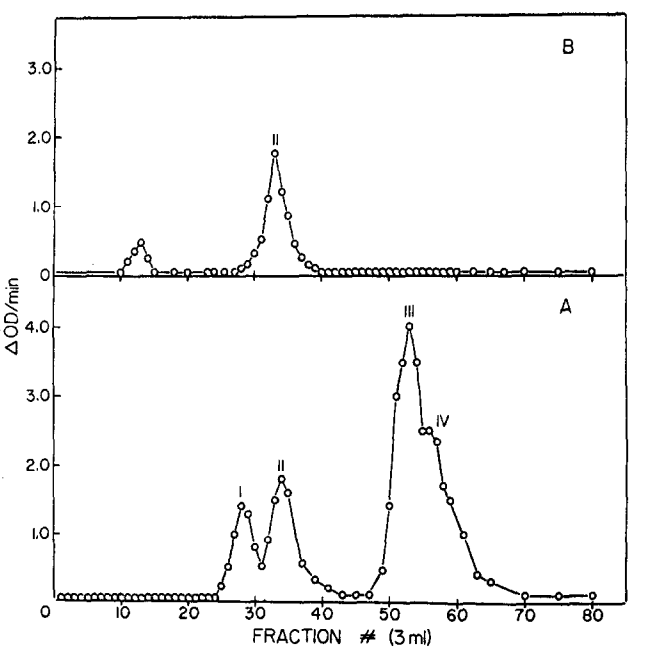

Fig. 2. A. DEAE-cellulose column elution pattern of a partially purified total homogenate from spinach leaves. Three distinct peaks plus a shoulder associated with peak III were obtained. B. Elution pattern of malic dehydrogenase isolated from purified chloroplasts. The major peak (II) corresponds exactly with peak II of the total homogenate. The first small peak apparently represents column overflow. Peak I is designated as a mitochondrial-malic dehydrogenase, peak II as a chloroplast-malic dehydrogenase and peaks III and IV as supernatant-malic dehydrogenases.

chondria also resulted in a single malic dehydrogenase band (not shown) which corresponded to the slowest malic dehydrogenase band of the total homogenate. The remaining 2 anodal bands were not associated with either chloroplasts or mitochondria and thus were designated as supernatant forms.

Elution of the partially purified homogenate from a DEAE-cellulose anion exchange column resulted in three distinct peaks plus a "shoulder" associated with the third peak (Fig. 2A). The extract obtained from the purified chloroplasts emerged from an identical column with an elution volume corresponding exactly with the second peak (Fig. 2B). Starch gel electrophoresis of the four peaks obtained from the column elution resulted in four distinct bands which corresponded to the electrophoretic bands obtained from the total homogenate (Fig. 1.-2 through 5). These data indicate that spinach leaves have four malic dehydrogenase isozymes; a mitochondrial-malic dehydrogenase, a chloroplast-malic dehydrogenase, and two supernatant-malic dehydrogenases. To our knowledge, this is the first report of a unique malic dehydrogenase protein associated with chloroplasts. In conformity with the previously described mitochondrial- and supernatant-malic dehydrogenases, the malic dehydrogenase associated with chloroplasts is designated as chloroplast-malic dehydrogenase (C-MDH).

The finding of a unique malic dehydrogenase protein associated with chloroplasts suggests several important questions concerning chloroplast metabolism and development. Because there is much evidence in the literature demonstrating that malate is a major product of photosynthesis (Calvin and Massini, 1952; Hatch and Slack, 1968), chloroplast-malic dehydrogenase is certainly an important chloroplast protein. Since chloroplasts are known to contain RNA and DNA (Gibor and Granich, 1964), it would seem important to know whether or not the chloroplast codes and synthesizes its own malic dehydrogenase.

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