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COEXISTENCE OF ISOCITRATE LYASE AND NADP-ISOCITRATE DEHYDROGENASE IN TURBATRIX ACETI MITOCHONDRIA

Michael P. McKinley and Richard N. Trelease
Departments of Zoology and Botany-Microbiology
Arizona State University
Tempe, Arizona 85281

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SUMMARY: Glyoxylate and Krebs cycle marker enzymes cobanded on sucrose gradients after isopycnic centrifugation of mitochondrial pellets. Mitochondrial heterogeneity was investigated by treating particles with a Nitro Blue Tetrazolium reaction mixture designed to specifically increase the buoyant density of mitochondria containing the Krebs cycle enzyme isocitrate dehydrogenase. The banding of Krebs cycle enzymes at a heavier buoyant density was accompanied by the glyoxylate cycle marker isocitrate lyase. Both isocitrate-metabolizing enzymes appear to be compartmented within the same mitochondria. This strongly suggests that regulation of one or both of these enzymes is necessary for directing carbon flow through the two different cycles.

The glyoxylate bypass is a Krebs cycle variant providing for the net synthesis of biosynthetic intermediates for organisms subsisting on acetate, ethanol or triglycerides (1-5). The enzymes of the bypass are typically compartmentalized within specialized organelles termed glyoxysomes (6). However, Rubin and Trelease (7) showed that mitochondria contained glyoxylate bypass enzymes in developing larvae of the parasitic nematode Ascaris suum. Recent work with a free-living form, Caenorhabditis elegans, suggested a particulate, non-mitochondrial compartmentation of these enzymes (8). McKinley and Trelease (9) subsequently found that in another free-living species, Turbatrix aceti, glyoxylate and Krebs cycle enzymes cobanded with mitochondria on sucrose gradients. The investigation reported here was undertaken to determine whether two separate groups of mitochondria banded together, i.e., one containing glyoxylate cycle markers and another housing Krebs cycle catalysts. The approach was to compare the distribution of isocitrate lyase (a glyoxylate cycle marker) and Krebs cycle enzymes on sucrose gradients after increasing the buoyant density of isocitrate dehydrogenase-containing mitochondria.

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MATERIALS AND METHODS

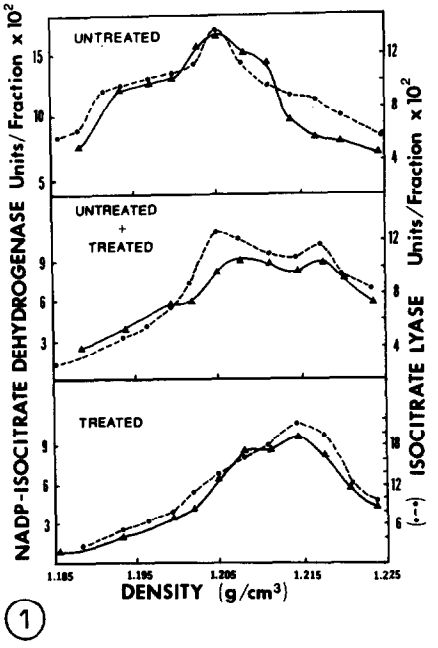
Axenic cultures of T. aceti were grown in a basal medium described by Rothstein (10). Fourteen-day-old worm cultures were harvested, cleaned and homogenized as previously described (9), except that the grinding medium contained 250 mM sucrose and 1 mM EDTA in 50 mM Tris-HCl, pH 8.5. Differential fractions were collected and handled as before (9), except that mitochondrial pellets were collected at 10,200 x g for 10 min. This pellet was either treated with Nitro Blue Tetrazolium prior to gradient application or it was layered untreated onto a 46-ml sucrose gradient (38 to 51% w/w) buffered with 50 mM Tris-HCl, pH 7.5. The particles were centrifuged to equilibrium in a Beckman SW-25.2 rotor for 13 hr at 29,000 x g. Two-ml fractions were collected dropwise from the bottom of cellulose nitrate tubes. Activities of the following enzymes were determined as described in the accompanying references: isocitrate lyase (EC 4.1.3.1) (9); succinate dehydrogenase (EC 1.3.99.1) (11); NAD-isocitrate dehydrogenase (EC 1.1.1.41) and NADP-isocitrate dehydrogenase (EC 1.1.1.42) (12).

Treatment of mitochondrial samples for NADP-isocitrate dehydrogenase reactivity was accomplished by incubating the particles for 20 min in the following Nitro Blue Tetrazolium reaction mixture: 50 mg Na^+-isocitrate, 5 mg Nitro Blue Tetrazolium, 5 mg NADP, 0.6 mg phenazine methosulfate, 1 ml 10 mM manganous chloride, 15 ml 500 mM Tris-HCl, pH 7.5 containing 250 mM sucrose, and 7.8 ml distilled water. The reaction mixture was prepared immediately prior to incubation of the organelles. Following incubation, particles were pelleted and resuspended in gradient buffer prior to application to the sucrose gradient.

Polyacrylamide gel electrophoresis was done according to Volk et al. (13). Isocitrate dehydrogenase reactivity was detected on gels by the method of Reeves et al. (14).

RESULTS AND DISCUSSION

Isopycnic centrifugation of untreated mitochondrial pellets yielded similar profiles of glyoxylate (isocitrate lyase) and Krebs cycle (NAD-, NADP-isocitrate dehydrogenase, and succinate dehydrogenase) enzyme activities peaking together at 1.204 g/cm^3 (Figs. 1 and 2, upper panels). Fumarase activities showed a similar distribution, but the data are not plotted. To select for Krebs cycle mitochondria only, particles were treated with the Nitro Blue Tetrazolium reaction mixture. The reaction produces an insoluble formazan (15) which should increase the buoyant density of the stained organelles. Figures 1 and 2 (lower panel) show that the Nitro Blue Tetrazolium-treated particles equilibrated at a higher density (1.214 g/cm^3) than the untreated controls (1.204 g/cm^3) (cf. lower panels to upper panels). The banding of NADP-isocitrate dehydrogenase activity at the higher density was coincident with



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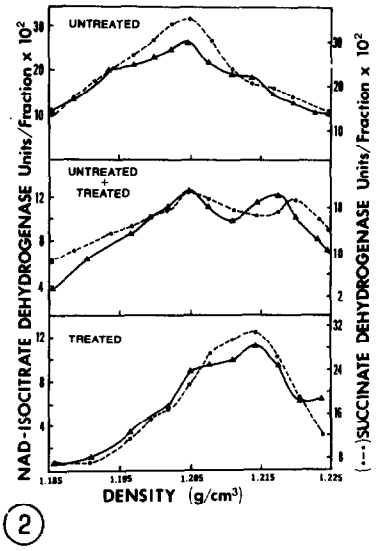

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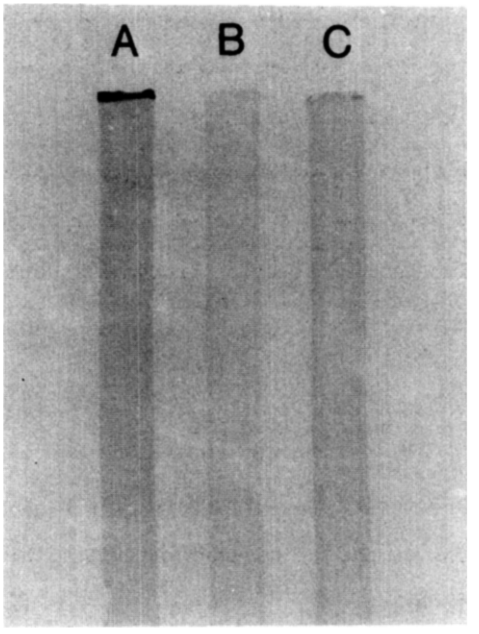
1. Distribution of isocitrate lyase and NADP-isocitrate dehydrogenase activities on equilibrium sucrose density gradients under three experimental conditions: UNTREATED - organelles applied to gradient immediately following isolation from organism, TREATED - organelles applied to gradient following incubation with Nitro Blue Tetrazolium reaction mixture, UNTREATED plus TREATED - a mixture of organelles from both of the above conditions applied to the gradient.

2. Distribution of succinate dehydrogenase and NAD-isocitrate dehydrogenase activities on equilibrium sucrose density gradients under the experimental conditions as described in Fig. 1: UNTREATED, TREATED and UNTREATED plus TREATED.

other Krebs-cycle enzymes. Of significance was the distribution of isocitrate lyase; it also peaked at the higher buoyant density (Fig. 1, lower panel). That the Nitro Blue Tetrazolium-treated mitochondria were not influencing the equilibration of unreacted mitochondria was shown in experiments where treated and untreated mitochondria were mixed and centrifuged (Figs. 1 and 2, middle panels). Glyoxylate and Krebs cycle enzymes co-banded in both the 1.204 and 1.214 g/cm^3 peaks.

Polyacrylamide gel electrophoresis was used to demonstrate the specificity of the Nitro Blue Tetrazolium treatment for NADP-isocitrate



3. Staining of NADP-isocitrate dehydrogenase activity on polyacrylamide disc gels. Electrophoresis and staining were as referenced in the Methods. Gel A - complete reaction medium, gel B - NADP omitted, gel C - isocitrate omitted. A single band showing enzyme reactivity occurs at the top of gel A only.

dehydrogenase. Figure 3 shows a single band at the top of gel A (complete reaction mixture), while the control gels B and C (lacking cofactor and substrate, respectively) remained unstained. That NADP-isocitrate dehydrogenase did not migrate into the gels was not unexpected; Seelig and Colman (16) reported a similar result with this enzyme extracted from heart tissue. Therefore, the equilibration of mitochondria at the heavier density must have been due to their isocitrate dehydrogenase reactivity, and since isocitrate lyase activity accompanied this shift, one is left to conclude that both enzymes are in the same mitochondria.

Reiss and Rothstein (17) found that isocitrate lyase exists as five isozymes which migrate 2 to 3 cm into polyacrylamide gels. The gels were scanned at 324 nm to detect the phenylhydrazone produced by the isocitrate lyase activities. We have confirmed these results using the system described

for electrophoresing the NADP-isocitrate dehydrogenase shown in Figure 3. The different migration of these two enzymes in the same gel system indicates that the two enzymes do not exist as an isocitrate-metabolizing complex, and therefore are probably not regulated as such. Isocitric acid catalysis within the two cycles is more likely regulated by another mechanism(s), perhaps involving the five isocitrate lyase isozymes. Work is in progress to elucidate this problem.

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