EMBRYOGENESIS AND THE GLYOXYLATE CYCLE

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Received 13 May 1980

1. Introduction

The key catalysts of the glyoxylate cycle, isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2), have been investigated in a variety of tissues [1–3]. In general, the biological assimilation of acetate units or the conversion of fatty acids to carbohydrates requires function of glyoxylate cycle [4–6]. For example, the function of this cycle is required for conversion of fat to carbohydrate during germination of fat-rich seedlings [7]. Moreover, the specific activities of isocitrate lyase and malate synthase are highest in second-stage larvae of the nematode *Ascaris lumbricoides* at a time when the conversion of lipid to carbohydrate is maximal [8].

The presence of isocitrate lyase and malate synthase has also been reported in several other nematodes [9,10] including a number of free-living forms [11–14]. Although in recent years scattered information has been published on the biochemistry of larval development of free-living nematodes such as *Turbatrix aceti* [14,15] and *Caenorhabditis elegans* [16], information about the basic biochemistry is still sketchy. For example, nothing is known about the function of glyoxylate cycle enzymes during embryogenesis, although the presence of high levels of isocitrate lyase in early post-embryonic states of *C. elegans* [16] implies that this enzyme functions during embryogenesis. We now present evidence which supports the function of the glyoxylate cycle during embryogenesis of *C. elegans*. As far as we know, this is the first report implicating the occurrence of the glyoxylate cycle in any embryonic animal tissue.

2. Materials and methods

Caenorhabditis elegans obtained from Dr R. L. Russell (Univ. Pittsburgh, Pittsburgh, PA) was grown monoxenically [11] in a liquid S-medium containing *E. coli* (strain K8-5m, CGSC strain no. 4868) for 2 weeks in two 500 ml cultures at 23°C on a rotary shaker at 120 rev./min. The bacterial strain is an isocitrate lyase-negative mutant.

Monoxenically-grown L1 larvae of *C. elegans* enter a semidormant quiescent state (dauerlarvae) if the *E. coli* feedstock is reduced [17,18]. Recovery to normal development can be induced by transferring the dauerlarvae to fresh medium with an adequate bacterial population.

Cultures of *C. elegans* were starved for 2 weeks at 23°C to produce dauerlarvae, each of the worm populations collected by centrifugation, and the two worm masses combined and treated with 1% sodium dodecyl sulfate (SDS) at 25° for 30 min. After washing with distilled water several times to remove SDS, the dauerlarvae were transferred into a 2-liter Erlenmeyer flask containing 500 ml sterile S-medium supplemented with freshly grown *E. coli* (1 g wet packed organisms/flask). The culture was shaken (120 rev./min) at 23°C and in 40–50 h larvae had resumed their normal growth and had matured into adults. At this stage the mature worms had 2–5 eggs. These worms were collected by centrifugation and washed twice with distilled water. The pelleted, washed worms were resuspended in 0.4 N NaOH, stirred slowly for 50 min at 4°C then subjected to a 10 min treatment at 4°C with 0.1 N NaOH containing 0.5% sodium hypochlorite. This combination of treatments digested the worms and released eggs. The eggs plus worm carcasses were washed by centrifugation several times with distilled water to remove hypochlorite and alkali, and the eggs floated in 0.88 M potassium phosphate (pH 7.0) removed with a Pasteur pipet, washed thrice by centrifugation aseptically, transferred into S-medium containing *E. coli*, and incubated at 23°C with shaking at 120 rev./min. The

entire washing sequence took 45 min. In parallel experiments, it has been established that 79% of the eggs hatch to release larvae synchronously as evidenced by the facts that all L1 larvae appeared between 9–12 h and 89% between 9–11 h [19].

After washing to remove *E. coli* [16] eggs (or larvae) were resuspended in 50 mM Tris–HCl (pH 7.6, 30°C) containing 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol and 0.2 mM α-toluenesulfonyl fluoride. About 50 g acid-washed sand was added to the suspension which was then subjected to sonic treatment at 4°C for 3 min at 60% of full power using a Biosonic II disintegrator from Bronwill Scientific, Rochester, NY. Homogenates were centrifuged at 10 000 × *g* for 15 min at 4°C, and the supernatant decanted yielding a clear fluid which was used without further purification. Isocitrate lyase, malate synthase, catalase and $NADP^+$-isocitrate dehydrogenase were assayed [20] at 30°C as was fumarase [21]. Triglyceride was estimated as in [22] and carbohydrate as in [23].

3. Results and discussion

The development of $NADP^+$-isocitrate dehydrogenase and fumarase in embryos is contrasted in fig.1A with that of isocitrate lyase and malate synthase (fig.1B) and catalase (fig.1A). These latter three activities increased rapidly during embryogenesis, and reached a maximum at 12 h by which eggs had hatched yielding L1 larvae. The enzyme profiles establish that isocitrate lyase, malate synthase and catalase appear during embryogenesis of *C. elegans*. The similar pattern of all three enzymes may be due to the particulate nature of these enzymes which are non-mitochondrial in cultures of *C. elegans* at random developmental stages [12]. Of considerable interest is the fact that the increase in isocitrate lyase and malate synthase correlates with a pronounced decrease in the triglyceride:carbohydrate ratio (fig.1B) calculated from the data presented in fig.1C. There was no change in the level of fumarase and isocitrate dehydrogenase, known mitochondrial markers, throughout the developmental period (fig.1A).

Although isocitrate lyase and malate synthase have been found in trematodes [25] and several species of free-living nematodes [12–15], there has been no published evidence that they participate in a functional glyoxylate cycle. This paper establishes that an increase in these two key catalysts in the glyoxylate cycle is correlated with lipid depletion and carbohydrate synthesis in *C. elegans* during embryogenesis. It is plausible that function of the glyoxylate cycle during embryogenesis occurs in other animals. In light of these observations it will be extremely important to probe for the function of isocitrate lyase and malate synthase in various developmental states of metazoans.

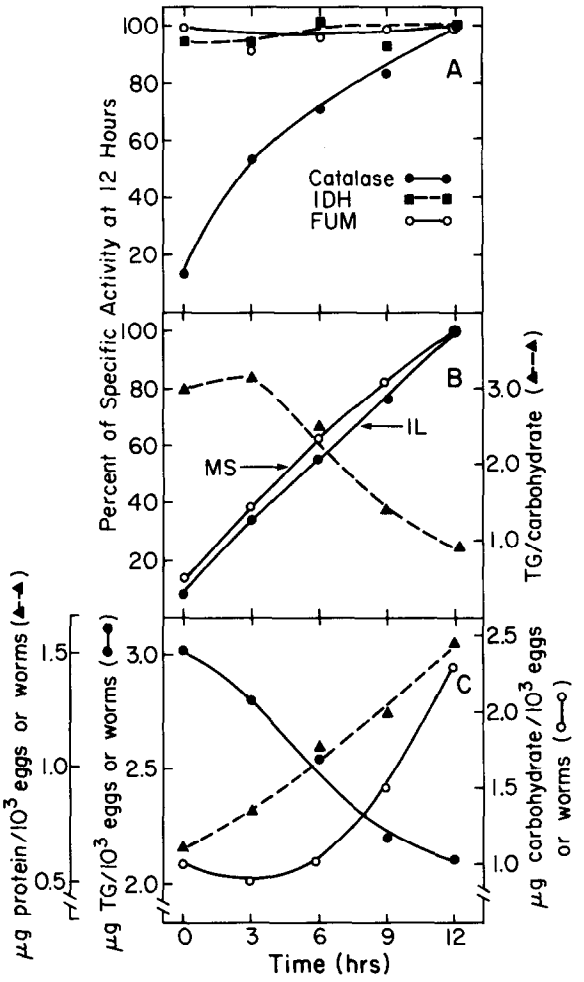

Fig.1. Changes during embryogenesis in levels of: catalase (●——●), fumarase, (○——○) and $NADP^+$-isocitrate dehydrogenase (IDH) (——) shown in (A); isocitrate lyase (IL) (●——●), malate synthase (MS) (○——○), and triglyceride (TG): carbohydrate ratio (in $\mu g/10^3$ eggs) (▲——▲) in (B); and protein, TG and carbohydrate in (C). Specific activities of IL, MS, catalase, fumarase and IDH were, respectively: 10.4, 15.6, 8.7 × 10^3, 420 and 680 nmol substrate disappearing (or product appearing) . min^{-1} . mg $protein^{-1}$ [24] at 12 h. Standards employed were glycerol for triglyceride and glucose for carbohydrate.

Acknowledgements

We thank D. Boehme for excellent technical assistance. This research was supported in part by National Science Foundation Grant PCM-7909786.

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