GLYCINE SYNTHESIS AND ISOCITRATE LYASE IN THE NEMATODE, CAENORHABDITIS BRIGGSAE*

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The metabolism of the nematode Caenorhabditis briggsae differs substantially from that expected of an "animal" species. This organism has at least a limited ability to synthesize "essential" amino acids (1) and, although it utilizes the tricarboxylic acid cycle, does not appear to have a typical cytochrome system (2). This paper presents evidence for the existence of another interesting metabolic feature, namely the presence of the enzyme "isocitrate lyase" and its utilization by C. briggsae to synthesize the amino acid, glycine. The existence of this pathway was first suspected when it was found that the organism excreted glycine as a major radioactive product after exposure to aspartate-4-C^{14} (Table I).

C. briggsae was grown under axenic conditions as previously described (3). Amino acids were isolated by ion exchange chromatography and their identities verified by paper chromatography and scanning for radioactivity (1). The identity of the glycine peak was further confirmed by two-dimensional paper chromatography followed by radioautography.

The fact that more activity is excreted in the form of synthesized products than is incorporated into the worms has been observed previously (2). The amount of isotope in the worm hydrolysate is too small to have any bearing on the relative activities of the excreted amino acids.

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TABLE I

Radioactivity Recovered in Amino Acids
After Incubation of C. briggsae with Aspartate-4-C^{14}

Time (Days)	Exp't*	Glutamate		Alanine		Serine		Glycine	
		counts/min	μ moles	counts/min	μ moles	counts/min	μ moles	counts/min	μ moles
3	1M**	11,600	3.0	5,800	2.33	280	3.4	6,100	.39
3	1W**	< 500		< 600		< 600		< 600	
7	2M†	3,730	0.27	5,220	1.2	1,237	0.04‡	18,900	‡

* M = medium; W = worm hydrolysate.

** Two flasks, each containing approximately 8.2×10^6 counts/min (7.7 μmoles) of aspartate-4-C^{14} in 4 ml of 0.12 M phosphate buffer, pH 7; (total number of worms, 730,000). Penicillin (400 units) and streptomycin (400 μg) were added.

† Conditions similar to experiment 1, but 4 flasks containing a total of 474,000 worms were used. The worm hydrolysate contained 5500 counts/min of which 2910 counts/min were in aspartic acid. The amount of radioactivity in the neutral amino acid fraction from Dowex-1 acetate was negligible.

‡ Too low to measure accurately.

The only reasonable explanation for these results seemed to be the existence of the following pathway: aspartate-4-C^{14} → oxalacetate-4-C^{14} → citrate-1-C^{14} → isocitrate-1-C^{14} → glyoxalate-1-C^{14} and succinate (by the action of isocitrate lyase) → glycine-1-C^{14} (by transamination). In essence, two carbon atoms go into the cycle as acetate and two (other) carbon atoms are removed as glycine.

Synthesis of glycine via serine is improbable in view of the relative specific activities of the two compounds. If such a route were involved, it would presumably involve the pathway: pyruvate → carbohydrate → glyceric acid or its phosphate → serine (4,5). However, pyruvate derived from aspartate-4-C^{14} should not be highly labeled; the low specific activity of the isolated alanine bears this out. Conclusive evidence against operation of this pathway was obtained by an experiment

in which C. briggsae was incubated with pyruvate-1-C^{14}. Both from the worm hydrolysate and from the medium, alanine was the most highly labeled compound (2.73×10^6 counts/min). Aspartate contained 1.54×10^5 counts/min, presumably being formed via CO_2 fixation and subsequent transamination of oxalacetate. Neither glycine nor serine were significantly labeled (8430 and 5000 counts/min respectively in the worms; essentially no activity in the medium).

As further evidence for the proposed route of glycine synthesis, isocitrate lyase was demonstrated directly in a number of experiments using the following procedure. Sonicates were prepared from approximately 1×10^6 axenically grown worms in 4 or 5 ml of water containing 1.6 m moles of potassium phosphate buffer, pH 7, using a Branson "Sonifier" at 0° C, followed by centrifugation in the cold for 10 min. at 600 X g. Experiments were performed in 25 ml Erlenmeyer flasks each containing phosphate buffer, pH 7, 200 μmoles; cysteine HCl, 6 μmoles; $MgCl_2$, 5 μmoles; $NaHSO_3$, 10 μmoles; sonicate, 1.6 ml. The bisulfite was added to trap any glyoxalate formed. To the experimental flask was added 8.1 μmoles of D,L sodium isocitrate. Total volume was 3 ml. Incubations were usually carried out at room temperature for periods of 30 or 40 minutes. Keto acid 2,4-dinitrophenylhydrazones were prepared, extracted and chromatographed quantitatively on paper using butanol - ethanol - 0.5 N NH_3, 70:10:20 (6). Two spots, R_f 0.21 and 0.53 respectively, matched exactly the spots from synthetic glyoxalate 2,4-dinitrophenylhydrazone (GDNP) which had been put through the same procedure. In two experiments, the amount of GDNP obtained from the experimental flask was clearly greater than that from the control. In an additional experiment, the GDNP spots were eluted from the paper; the spectra in 95% ethanol matched exactly those of authentic products when compared in a Cary 14 R Spectrophotometer. The quantitative results are shown in Table II.

TABLE II

Optical Density of Glyoxalate 2,4-Dinitrophenylhydrazones (GDNP's)

Experiment	GDNP I (360 mμ)	GDNP II (367 mμ)*
Control 1	.10	.28
Experiment 1	.19	.43
Control 2	.10	.30
Experiment 2	.22	.42

* The isocitric acid, when carried through the experimental procedure, yielded a yellow spot in the area of GDNP II, with a peak at 275 mμ. Values of GDNP II have been adjusted accordingly.

The identity of the GDNP's was further confirmed by comparison of the spectra in 1 N NaOH with authentic material. The GDNP's were also checked against authentic material on paper chromatograms in two other sets of solvents.

Although the enzymes of the glyoxalate cycle, malate synthetase and isocitrate lyase have been found in numerous bacterial species, in plants, and in the protozoan Tetrahymena pyriformis (7) they do not appear to be found in mammalian tissues or insects (8). The present report, in fact, appears to be the first demonstration of isocitrate lyase in a multicellular animal species. Time course studies with glyoxalate-C^{14} both in intact worms and worm sonicates have failed to demonstrate the direct synthesis of malate. If the absence of malate synthetase holds true, it would suggest that the isocitrate lyase is present in C. briggsae for the purpose of glycine synthesis. Such a pathway has been proposed for microorganisms but experimental proof of its magnitude is lacking. The pathway is not of major importance in Penicillium chrysogenum (9).

Acetate has previously been shown to yield glycine (which can be converted to serine) (1). It remains to be seen if this is the major route of glycine synthesis in C. briggsae, or if the pathway depends upon the substrate. It is quite possible that if carbohydrate is involved, synthesis of glycine via serine may predominate.

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