Proceedings of the Iowa Academy of Science

Volume 81 | Number Article 7

1974

Detection of Isocitrate Lyase in Achlya flagellata

Joanne Tontz Ellzey
University of Texas at El Paso

Christina D. Chavez
University of Texas at El Paso

Detection of Isocitrate Lyase in *Achlya flagellata*

JOANNE TONTZ ELLZEY and CHRISTINA D. CHAVEZ[1]

ELLZEY, JOANNE TONTZ, and CHRISTINA D. CHAVEZ (Dept. of Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968). Detection of Isocitrate Lyase in *Achlya flagellata*. *Proc. Iowa Acad. Sci.* 81(1): 10-11, 1974.

The colorimetric procedure of McFadden and Howes (1960) and the enzyme assay for isocitrate lyase (Shiio, Shiio, and McFadden, 1965) gave positive results for the presence of isocitrate lyase in *Achlya flagellata* oogonial cultures. This enzyme is an indicator of the glyoxylate pathway (metabolism of lipids to carbohydrates). Further work is in progress to determine if there is a correlation between isocitrate lyase and the microbodies of oogonial initials.

INDEX DESCRIPTORS: Isocitrate Lyase, *Achlya flagellata*, Achlya Isocitrate Lyase.

The glyoxylate cycle couples fatty acid degradation with carbohydrate synthesis. C_2 compounds enter the citric acid cycle as acetyl-CoA, which condenses with oxaloacetic acid, producing citric acid. The citric acid is converted to isocitric acid. The key enzymes of the glyoxylate cycle are (1) isocitrate lyase, which catalyzes the conversion of isocitrate $\longleftrightarrow$ succinate and glyoxylate and (2) malate synthase, which catalyzes the reaction, acetyl coenzyme A and glyoxylate $\rightarrow$ malate (Dixon and Kornberg, 1959).

Reports of isocitrate lyase in fungi have been primarily concerned with the higher fungi (Kornberg and Collins, 1958; Collins and Kornberg, 1960; Gottlieb and Ramachandran, 1960; Heberling, Berky, and Stone, 1960; Frear and Johnson, 1961; Turian, 1961; Duntze et al., 1969; Kohr, Vanderhaeghe, and Combépine, 1969; Szabo and Avers, 1969; Cotter et al., 1970; Perlman and Mahler, 1970; Selem and Sawsan, 1970; O'Sullivan and Casselton, 1973). McCurdy and Cantino (1960) discovered a metabolic route from glyoxylate to glycine in the chytridiomycete *Blastocladiella emersonii*. The following paper is the first report of the glyoxylate pathway in the oomycetes.

In unpublished ultrastructural data, Ellzey observed the association of microbodies with storage bodies. The contents of the storage bodies appeared to be converted from lipid to carbohydrate in *Achlya flagellata* Coker oogonial initials. Therefore, an assay was performed to determine if isocitrate lyase was present in oogonial cultures of *A. flagellata*.

MATERIALS AND METHODS

Pure cultures of *Achlya flagellata* (ATCC #14566) were grown on autoclaved hemp seeds in sterile water, as well as on Barksdale Medium #5, containing 400 mg edamin, 2400 mg glucose, 80 mg calcium glycerophosphate, 1 ml 0.5 M $MgSO_4 \cdot 7H_2O$, 0.5 ml 2.0 M KCl, 0.5 ml metal mix #4, and 100 mg Tris in 1000 ml distilled water (pH 6.8). After autoclaving, 400 mg of potassium bicarbonate in 10 ml of distilled water was added to Barksdale Medium #5. Five-day-old cultures containing hyphae, oogonia, antheridia, and oospores were harvested by centrifugation at 15,000 rpm for 15 min. A mortar and pestle were used to disrupt the fungal walls in 0.05 M Tris buffer containing 0.05 mM $MgCl_2$. After

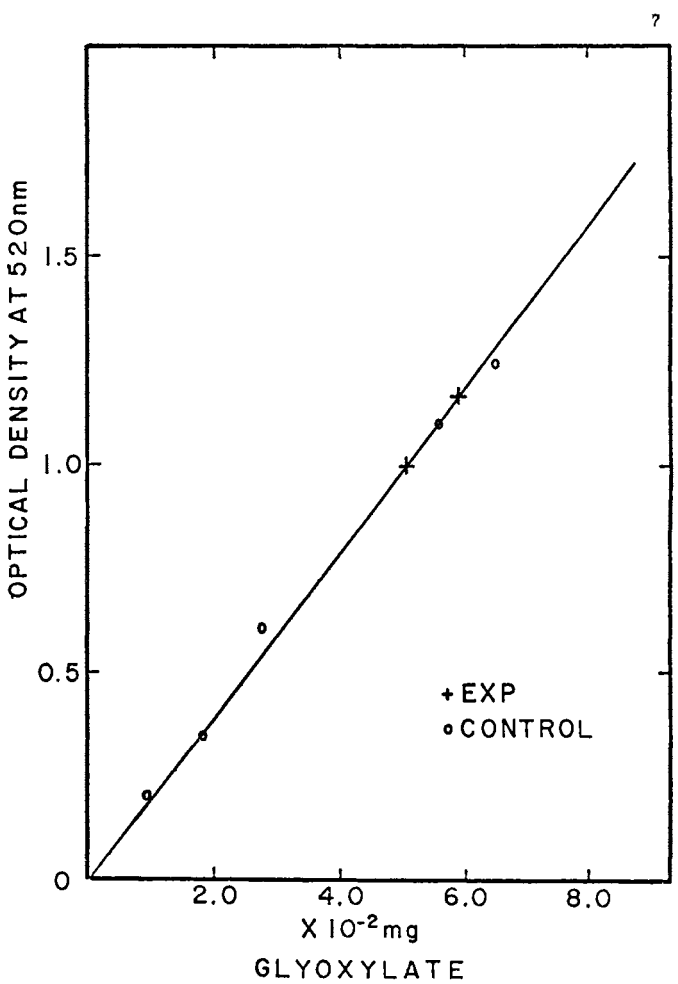

Figure 1. Optical density at 520 nm as a function of glyoxylate concentration in 5 ml of solution.

[1] Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968.

centrifugation at 15,000 rpm for 15 min, the supernatant from 1 gm wet wt. of fungus was assayed for isocitrate lyase, using the colorimetric procedure of McFadden and Howes (1960) and Shiio, Shiio, and McFadden (1965). The supernatant was preincubated with 55 μmoles of glutathione for 10 min at room temperature in a total volume of 5 ml containing 455 μmoles of Tris and 13.6 μmoles of $MgCl_2$. To initiate the enzymatic reaction, 0.45 ml of 0.04 M sodium DL-isocitrate was added and allowed to react for 10 min at 30° and 20 min at room temperature. The reaction was stopped with the addition of 2.3 ml of 10% (w/v) trichloracetic acid. The fungal reaction mixture was assayed for the formation of glyoxylic acid phenylhydrazone from glyoxylate, an endproduct of

$$\text{isocitrate} \xleftarrow{\text{isocitrate lyase}} \text{succinate and glyoxylate}$$

and phenylhydrazine hydrochloride.

The control consisted of a 5 ml solution containing 0.025-0.2 μmoles glyoxylate. One ml of 1 M oxalic acid buffer at pH 1 and 1.0 ml of 1% phenylhydrazine HCl were added to the control. This solution was boiled and then cooled for 5 min. The control was then chilled in an ice bath to room temperature. Concentrated HCl (4 ml) and 5% potassium ferricyanide (1.0 ml) were added. After thorough mixing, the percent transmission vs. μmoles of glyoxylate was determined using a Bausch and Lomb Spectronic 20 colorimeter set at 520 nm. This wavelength was chosen after plotting a spectrum for the red-violet colored product of oxidized glyoxylic acid phenylhydrazone and selecting the peak absorbancy. The same procedure was followed for 5 ml of the fungal supernatant.

RESULTS AND DISCUSSION

Figure 1 compares the optical density of the control containing 0.025-0.2 μmoles glyoxylate/5 ml solution with the optical density of 5 ml fungal supernatant. A red-violet product was obtained from five-day-old cultures of *A. flagellata* grown on either hemp seeds or Barksdale Medium #5. Experimental solutions had 9.8 and 6.7% transmission. These readings were within the control range of 2.2-64.5% transmission.

The positive assay for isocitrate lyase in *A. flagellata* indicates the presence of the glyoxylate pathway. Further work is in progress to determine if microbodies of oogonial initials contain isocitrate lyase. Ultimately, we seek to determine if storage bodies of oogonial initials are the sites of conversion of lipids to carbohydrates.

REFERENCES CITED

COLLINS, J. F., and H. L. KORNBERG. 1960. The metabolism of C_2 compounds in micro-organisms. *Biochem. J.* 77: 430-438.

COTTER, D. A., A. J. LACLAVE, W. S. WEGENER, and D. J. NEIDERPRUEM. 1970. CO_2 control of fruiting in *Schizophyllum commune:* Noninvolvement of sustained isocitrate lyase derepression. *Can. J. Microbiol.* 16: 605-608.

DIXON, G. H., and H. L. KORNBERG. 1959. Assay for key enzymes of the glyoxylate cycle. *Biochem. J.* 72: 3P.

DUNTZE, W., D. NEUMANN, J. M. GANCEDO, W. ATZOPODIEN, and H. HOLZER. 1969. Studies on the regulation and localization of the glyoxylate cycle enzymes in *Saccharomyces cerevisiae. European J. Biochim.* 10: 83-89.

FREAR, D. S., and M. A. JOHNSON. 1969. Enzymes of the glyoxylate cycle in germinating uredospores of *Melampsora lini* (pers.) lév. *Biochem. Biophys. Acta* 47: 419-421.

GOTTLIEB, D., and S. RAMACHANDRAN. 1960. The nature of production of the glyoxylate pathway enzymes in germinating spores of *Penicillium oxalicum* (F-56). *Mycologia* 52: 599-607.

HEBERLING, R. L., J. J. BERKY, and R. W. STONE. 1960. Dissimilation of citric acid by extracts of *Penicillium chrysogenum. Arch. Biochem. Biophys.* 86: 102-109.

KOBR, M. J., F. VANDERHAEGHE, and G. COMBEPINE. 1969. Particulate enzymes of the glyoxylate cycle in *Neurospora crassa. Biochem. Biophys. Res. Comm.* 37: 640-645.

KORNBERG, H. L., and J. F. COLLINS. 1958. The glyoxylate cycle in *Aspergillus niger. Biochem. J.* 68: 3P-4P.

MCCURDY, H. D., JR., and E. C. CANTINO. 1960. Isocitratase, glycine-alanine transaminase, and development in *Blastocladiella emersonii. Plant Physiol.* 35: 463-476.

MCFADDEN, B. A., and W. V. HOWES. 1960. The determination of glyoxylic acid in biological systems. *Anal. Biochem.* 1: 240-248.

O'SULLIVAN, J., and P. J. CASSELTON. 1973. The subcellular localization of glyoxylate cycle enzymes in *Coprinus lagopus* (sensu Buller). *J. Gen. Microbiol.* 75: 333-337.

PERLMAN, P. S., and H. R. MAHLER. 1970. Intracellular localization of enzymes in yeast. *Arch. Biochem. Biophys.* 136: 245-259.

SELEM, K. G., and A. E. SAWSAN. 1970. The presence of isocitrate lyase in *Aspergillus niger. J. Microbiol. UAR.* 3: 233.

SHIIO, I., T. SHIIO, and B. A. MCFADDEN. 1965. Isocitrate lyase from *Pseudomonas indigofera.* I. Preparation, amino acid composition and molecular weight. *Biochim. Biophys. Acta* 96: 114-122.

SZABO, A. S., and C. J. AVERS. 1969. Some aspects of regulation of peroxisomes and mitochondria in yeast. *Ann. N. Y. Acad. Sci.* 168: 302-312.

TURIAN, G. M. 1961. L'acétate et son double effet d'induction isocitratasique et de differenciation conidienne chez les *Neurospora. C. R. Acad. Sci.* (Paris), ser. D, 252: 1374-1376.