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A COMPARISON OF MYO-INOSITOL-1-PHOSPHATE SYNTHASE ACTIVITY IN EUGLENA GRACILIS AND IN A BLEACHED MUTANT

M. W. LOEWUS[1], J. A. SCHIFF[2] and F. A. LOEWUS[1]

[1] Institute of Biological Chemistry, Washington State University, Pullman, WA 99164 USA
[2] Institute for Photobiology, Brandeis University, Waltham, MA 02254 USA

SUMMARY

1L-*myo*-Inositol-1-phosphate synthase, EC 5.5.1.4, was extracted from light- and dark-grown *Euglena gracilis* cells and a bleached mutant (W_3BUL). Within experimental error no differences in synthase activity were detected among these three cell types.

1. INTRODUCTION

1L-*myo*-Inositol-1-phosphate synthase (EC 5.5.1.4) has been isolated from plant and animal tissues (LOEWUS & LOEWUS 1983) and would be expected to occur in any eukaryotic tissue that did not require exogenous *myo*-inositol (MI) as a growth factor since MI is a component of phospholipids in all tissues (POSTERNAK 1962). Despite considerable research on the synthase, its intracellular localization is largely unexplored. The enzyme remains soluble in plant extracts at 43,000g (LOEWUS & LOEWUS 1971) and in animal extracts at 100,000g (EISENBERG 1965). IMHOFF & BOURDU (1973) found the synthase in isolated pea chloroplasts which prompts the question of whether the synthase is uniquely associated with that organelle.

Recently, GUPTA et al. (1984) partially purified the synthase from *Euglena gracilis* and described some of its properties but provided no information concerning subcellular localization. *E. gracilis* is used extensively in photobiology as a model for studies of chloroplast development (SCHIFF & SCHWARZBACH 1982) and useful mutants, among them the bleached mutant W_3BUF (OSAFUNE & SCHIFF 1980), have been isolated. It is of interest to compare the MI-1-P synthase of bleached mutant W_3BUF which contains only rudimentary proplastids with activities obtained from dark-grown wild type cells which contain proplastids and from light-grown cells which contain fully developed chloroplasts. If the synthase is uniquely associated with chloroplasts, the activity of this enzyme would be expected to be high in light-grown cells, low or undetectable in dark-grown cells, and even less in the bleached mutant.

2. MATERIALS AND METHODS

Euglena gracilis Klebs var. *bacillaris* Cori, wild type and the W_3BUF mutant, were grown in Hutner pH 3.5 medium (GREENBLATT & SCHIFF 1959). Light-grown cells were illuminated with white and red fluorescent lamps (HOLOWINSKY & SCHIFF 1970). Cells were harvested at late log phase, washed once in 0.05 mM Tris.HCl, pH 8, containing 0.5 mM dithiothreitol and frozen for 1 to 2 days prior to extraction.

Thawed cells were disrupted by sonication for 90 seconds at 7 mHz. The suspension was centrifuged at 20,000g, 4 °C, to yield a pellet and clear supernatant. Protein was precipitated with ammonium sulfate, 50 g/100 ml, at 0 °C, taken up in 10 ml of buffer and dialyzed 2 hours against 500 ml of buffer with one buffer change. Crude extracts were unsuited for testing due to differences in pH.

Synthase was assayed (LOEWUS & LOEWUS 1971) with the following modification. Effluent from the ion exchange column was concentrated and applied to Whatman no. 1 paper for chromatography in methanol – formic acid – water, 80: 15:5 by volume, for 8 hours. The MI spot, as determined by accompanying controls, was cut out and assayed while still on paper for radioactivity in scintillation fluid composed of 5.5 g of Permablend (Packard Intrument Co., Downers Grove, IL) dissolved in 1 liter of toluene. An aliquot was eluted from the paper and further identified by thin layer chromatography on cellulose in ethyl acetate – pyridine – water, 10:6:5 by volume, accompanied by appropiate controls. When a standard sample of MI was assayed, recovery was 99 to 100%.

Protein was recovered from extracts by precipitation with 5% trichloroacetic acid and analyzed by the Lowry procedure (LAYNE 1957).

A unit of synthase activity is defined as formation of 1 μmole of MI-1-P per minute.

3. RESULTS AND DISCUSSION

No significant differences in total synthase activity on a wet weight basis of cells were found in cells of wild type (light- or dark-grown) or the bleached mutant of *E. gracilis* (*table 1*). Differences in specific activity were due to extraction of more non-specific protein from light-grown cells. In preliminary studies, differences in specific activities due to less efficient extraction of protein from light-grown cells was also encountered. For three sets of extractions, the average recoveries of protein (with standard deviations) were 14.3 $\pm$ 4.6, 18.5 $\pm$ 7.8 and 19.4 $\pm$ 2.3 mg protein/g wet weight for cells of light-grown, dark-grown and the mutant respectively. In *E. gracilis*, MI-1-P synthase does not appear to be uniquely associated with the chloroplasts.

GUPTA et al. (1984) reported a specific activity of 1.34 mU/g fresh weight of cells for the crude 8,500g supernatant from light-grown cells of the Z strain of *E. gracilis* an activity somewhat greater that the value of 0.5 obtained for the dialyzed ammonium sulfate fraction used in the present study, possibly due

Table 1. *myo*-Inositol-1-phosphate synthase in *Euglena gracilis*

Sample	Protein mg/g wet wt. cells	Specific activity* mU/mg of protein	Total activity mU/g wet wt. cells
Light-grown cells	16.9		
Fresh extract		0.033 ± 0.002	0.56
Extract stored six weeks		0.029 ± 0.003	0.49
Dark-grown cells	22.9		
Fresh extract		0.024 ± 0.001	0.55
Extract stored six weeks		0.020 ± 0.002	0.46
W_3BUL mutant cells	19.4		
Fresh extract		0.026 ± 0.000	0.50
Extract stored six weeks		0.026 ± 0.001	0.50

* with standard deviations

to differences in the strain of *E. gracilis* used. They also reported complete inactivation of *E. gracilis* synthase by 5 mM ammonium sulfate. Unlike preparations from animal tissues (BARNETT & CORINA 1968), the ammonium sulfate fractionated activity from *E. gracilis* is stable for at least 6 weeks when stored at -20 °C. The specific activities per g of cells reported by GUPTA et al. and those found in the present study are in the range of values reported for synthase from *Lilium longiflorum* pollen (LOEWUS & LOEWUS 1974) and substantially higher than values from vegetative tissues (LOEWUS & LOEWUS 1971). This would suggest that *E. gracilis* is an excellent source for extraction and purification of this synthase.

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