DIFFERENTIAL RESPONSES OF TWO CARBOXYLASES FROM *EUGLENA* TO THE STATE OF CHLOROPLAST DEVELOPMENT

Alex EVERS and Mary Lou ERNST-FONBERG
Biology Department, Yale University, New Haven, Connecticut 06520, USA

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1. Introduction

Regulation of malonyl CoA substrate levels by modification of acetyl CoA carboxylase levels in response to lipid requirements, and through its allosteric properties, is accepted as a means of controlling fatty acid biosynthesis in animals [1]. Large changes in lipid composition [2] and fatty acid synthetase enzymology [3–5] are observed during chloroplast development in *Euglena*, but the acetyl CoA carboxylase activity of the organism had not been measured in the different developmental stages. Another carboxylase in *Euglena*. ribulose 1,5-diphosphate carboxylase, has been implied to vary in activity relative to chloroplast development [6]. The levels of both carboxylases were measured in conjunction with the stage of chloroplast development in *Euglena gracilis* strain Z, variety *bacillaris*, and mutants of the latter, Y_3BUD and W_3BUL [7]. The acetyl CoA carboxylase activity levels remained constant regardless of the stage of or capacity for chloroplast development. Alternatively levels remained constant regardphate carboxylase varied markedly depending on the capacity of the mutants to develop chloroplasts, as well as on the presence or absence of chloroplasts in the wild type cells.

2. Materials and methods

Cells were grown in the dark [4] or photoauxotrophically [8] in 1 liter cultures. Harvested cells were stored at −50°C. Enzyme extracts were prepared by suspending the cells in an equal volume of pH 7.0, 0.1 M imidazole buffer, 0.007 M in 2-mercaptoethanol. Aliquots were serially diluted with 0.86%NaCl and counted in a model B Coulter Counter. The remainder was purged with N_2 and disrupted with ultrasound. The mixture was centrifuged at 100 000 *g* for 1 hr, and the supernatant was brought to 60% saturation with ammonium sulfate. After 20 min, the precipitated protein was collected by centrifugation at 12 100 *g* for 15 min and dissolved in pH 7.0, 0.02 imidazole buffer, 0.007 M in 2-mercaptoethanol to the volume of the original supernatant. The protein solution was dialyzed against 10 vol of the same buffer for 1 hr then assayed for enzyme activity and protein [9].

Enzyme assays were done in glass liquid scintillation counting vials anchored in a 30°C circulating water bath. The vials were closed with serum caps after initiation of reaction and were linked in series through inlet and outlet 18 gauge needles inter-connected with tygon tubing. Both assays, final volume 0.25 ml, contained: $NaH^{14}CO_3$, 25 mM (specific activity 0.2); bovine serum albumen, 20 μg; pH 7.5 imidazole, 0.1 M; $MgCl_2$, 0.008 M; dithiothreitol, 0.002 M; and enzyme, 25–50 μl. In addition, the assay for acetyl CoA carboxylase contained ATP, 4 mM and acetyl CoA, 0.24 mM while the ribulose, 1,5-diphosphate, carboxylase assay contained ribulose 1,5-diphosphate, 0.68 mM. After a 15 min reaction period, 0.1 ml of 3 N HCl was injected, and air was circulated through the system to remove unreacted $^{14}CO_2$ which was trapped in 40% KOH. The solutions were dried at 55°C in the air current. Distilled water 0.1 ml, ethanol, 3 ml and 15 ml of toluene containing 15.1 g/gal of 2,5-diphenyloxazole were added to each vial, and radio-activity was measured in a Packard Model 3320 liquid scintillation counter. Quenching was corrected by the channels ratio method.

3. Results

Ribulose 1, 5-diphosphate carboxylase levels are shown in fig. 1. The activity per cell of dark-grown wild type cells was roughly 5–10 times higher than that of the chloroplast mutant Y_3, whereas the mutant W_3 yielded no detectable enzyme activity. The photoauxotrophically grown *Euglena* contained the largest amount of ribulose 1,5-diphosphate carboxylase per cell, roughly 10 times more than the corresponding dark grown organisms. The relative specific activities (enzyme units/mg protein) paralleled the activity per cell measurements in all cases.

Acetyl CoA carboxylase activity per cell (fig. 2) remained practically constant among the mutants and two wild types of *Euglena* and was independent of chloroplast development. Again, the specific activity of each culture paralleled the activity per cell measurements.

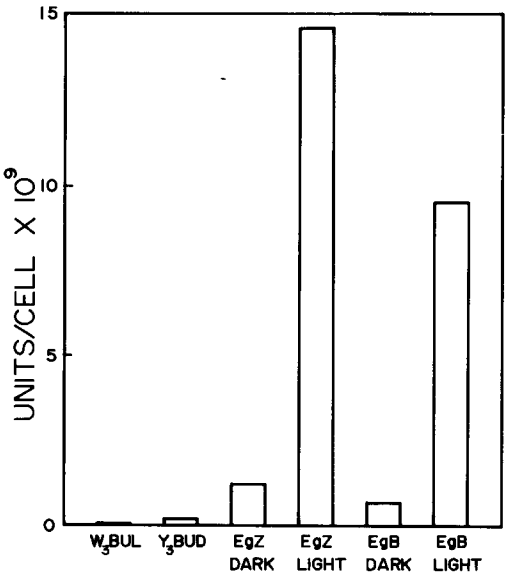

Fig. 1. Ribulose Diphosphate carboxylase activity, units/cell. A unit of activity was the incorporation of 1 μmole of $^{14}CO_2$/15 min. Data for each cell type were obtained from 3 separate cultures, and at least 3 separate assays were done on material from each culture. The data were averaged, and the results are shown in the figure, Details of the assay are given in the text. Values varied among cultures of the same organism to the extreme of a factor of 2. The designation EgB and EgZ indicate *Euglena Gracilis* variety *bacillaris* and strain Z respectively. W_3 BUL and Y_3 BUD are chloroplast mutants of variety *bacillaris*.

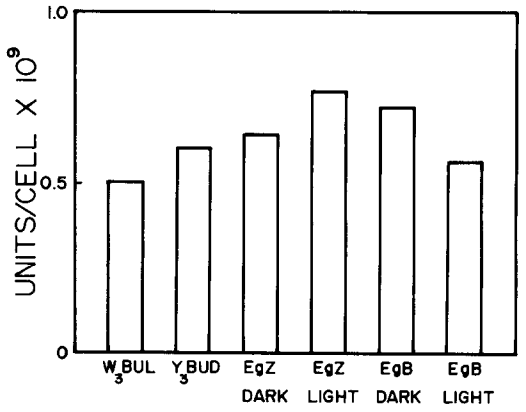

Fig. 2. Acetyl CoA carboxylase activity, units/cell. Units of activity and handling of the data were the same as that described under fig. 1.

4. Discussion

The levels of ribulose 1,5-diphosphate carboxylase varied depending on the capacity to form chloroplasts as well as with the presence or absence of chloroplasts in the wild type cells. The mutant Y_3 BUD contains plastid DNA and is capable of forming rudimentary chloroplast structures, whereas the W_3 BUL mutant lacks detectable chloroplast DNA and is incapable of forming rudimentary chloroplast structures [10]. Ribulose 1,5-diphosphate carboxylase from higher plants is composed of two non-identical subunits. The larger subunit is encoded on chloroplast DNA [11], and the smaller originates is nuclear DNA [12]. Therefore, the W mutant would be expected to be unable to synthesize intact enzyme if the biosynthetic origin of the enzym in the phytoflagellate is similar to that in higher plants.

Activation or induction of the acetyl CoA carboxylase does not appear to be a significant point of control of fatty acid biosynthesis in *Euglena*, and the chloroplast probably does not play any major role in conjunction with this enzyme in *Euglena*. Conversely, the fatty acid synthetase enzymology does vary in response to the stage of and capacity for chloroplast development [4–5] (Ernst-Fonberg, M. L., manuscript in preparation). An alternative means of controlling the availability of the substrates for fatty acid biosynthesis, malonyl CoA and NADPH, in this organism has been suggested by the demonstration of a multi-enzyme

complex of phosphoenolpyruvate carboxylase, malate dehydrogenase, and acetyl CoA carboxylase (Wolpert, J. S. and Ernst-Fonberg, M. L., manuscript in preparation). The complex is proposed to operate in conjunction with enzyme to provide a means of controlling the levels of substrates (malonyl CoA and NADPH) for fatty acid biosynthesis. On this basis, control exercised through variation in acetyl CoA carboxylase levels would not be expected and was not found.

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