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FINAL TECHNICAL REPORT

Project Title: Carbon and Hydrogen Metabolism of Green Algae
in Light and Dark

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In this final technical report, results of our studies on the following research projects with the green algae are presented.

1. chloroplast respiration
2. photoregulation of chloroplast respiration
3. reductive carboxylic acid cycle
4. oxy-hydrogen reaction

Chloroplast Respiration

In contrast to higher plants, little is known about carbohydrate respiratory pathways and its compartmentation in the unicellular green algae. This laboratory was the first to develop a method for isolation of an algal chloroplast in order to investigate metabolic events in a cellular component.

Chloroplastic respiration was monitored by measuring $^{14}\text{CO}_2$ evolution from labeled glucose in the absence and presence of various inhibitors. The patterns of CO_2 evolution were those expected from the oxidative pentose phosphate cycle and glycolysis. Comparing the inhibition of CO_2 evolution generated by pH 7.5 with respect to pH 8.2 (optimum) in chloroplasts given C-1, C-2, and C-6 labeled glucose indicated that a suboptimum pH affects the recycling of the pentose phosphate intermediates to a greater extent than CO_2 evolution from C-1 of glucose. This inhibition was alleviated by stromal alkalating agents. We conclude that the site which primarily mediates glucose respiration in the darkened algal chloroplast is the fructose-1,6-bisphosphatase/phosphofructokinase junction.

The respiratory pathway, we have described, can account for the total oxidation of a hexose to CO_2 and for interactions between carbohydrate metabolism and the oxyhydrogen reaction in algal cells adapted to a hydrogen metabolism.

The role of hexokinase in carbohydrate metabolism in the isolated algal chloroplast was studied by an external supply of labeled fructose, glucose, mannose, galactose, maltose, and ribose. There was an appreciable increase in the rate of CO_2 evolution in the presence of added ATP. The most noticeable increase was found with glucose. Probing the outer membrane polypeptides of the intact chloroplast with two proteases, trypsin and thermolysin, decreased CO_2 release from glucose. Gas release from glucose-6-phosphate was unaffected by treatment with trypsin.

To account for the residual glucokinase after treatment with trypsin, we suggest two pools of hexokinase exist, one in the stroma and one bound to the cytosolic side of the envelope.

We also evaluated the role of an electron transport pathway associated with aerobic carbohydrate degradation in the *Chlamydomonas* chloroplast. This was accomplished by monitoring the evolution of CO_2 from darkened chloroplasts externally supplied with labeled sugar in the presence of conventional electron transport inhibitors. Gas evolution was inhibited 30% by 50 micromolar rotenone and by amytal but

at 500- to 1000-fold concentrations indicating the involvement of a NADPH-plastoquinone reductase. CO_2 release was sensitive to propyl gallate and sacicyhydroxamic acid, both well-established inhibitors of cyanide-insensitive branch of mitochondrial-mediated respiration. Antimycin A (100 micromolar) and sodium azide (75 micromolar) inhibited approximately 80% and 50%, respectively. These results are interpreted as evidence for a respiratory electron transport chain functioning in the darkened, isolated chloroplast. Chloroplast respiration defined as CO_2 release from externally supplied glucose can account for at least 10% of total cellular respiration.

Photoregulation of Chloroplast Respiration

The photoregulation of chloroplastic respiration was monitored in darkness and in light by measuring labeled CO_2 release from whole chloroplasts of Chlamydomonas reinhardtii supplied externally with radioactive glucose and fructose. CO_2 release was inhibited more than 90% at a low light intensity of 4 watts per square meter. Oxidants such as oxaloacetate and phenazine methosulfate reversed the inhibition. The onset of the photoinhibitory effect on CO_2 release was relatively rapid compared to the restoration of CO_2 release following illumination. In the darkened chloroplast, dithiothreitol inhibited release. Of the four enzymes (fructokinase, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and gluconate-6-phosphate dehydrogenase) in the pathway catalyzing release of CO_2 , only glucose-6-phosphate dehydrogenase was deactivated by light and by dithiothreitol.

The Reductive Carboxylic Acid Cycle in a Green Alga

The reductive carboxylic acid cycle first reported in the photosynthetic green sulfur bacteria is in effect a reversal of the oxidative citric acid cycle of Krebs, and one complete cycle yields one molecule of oxaloacetic acid from four molecules of CO_2 . In this pathway, reduced ferredoxin is needed to form pyruvate from acetyl-CoA and CO_2 and alpha-ketoglutarate from succinyl-CoA and CO_2 catalyzed by pyruvate synthase and alpha-ketoglutarate synthase, respectively.

Inasmuch as there are no reports in the literature to support the existence of the cycle in a eukaryotic cell, the F-60 mutant of the green alga, Chlamydomonas reinhardtii, seemed appropriate to probe for this pathway because this cell lacks an intact Calvin-Benson cycle and acetate enhances CO_2 fixation by this cell.

Evidence was obtained consistent with the presence of the reductive cycle in Chlamydomonas. This conclusion is based on the fact that: (a) acetate roughly doubled CO_2 fixation in whole cells and in isolated chloroplasts; and (b) pyruvate synthase, alpha-ketoglutarate synthase, and ATP-citrate lyase, three indicators of the cycle, were found in cell-free extracts.

The Oxyhydrogen Reaction

This reaction first noted by Hans Gaffron about 50 years ago involves the simultaneous uptake of hydrogen gas and oxygen in the darkened algal cell adapted to a hydrogen metabolism. Under

oxyhydrogen conditions, the chemosynthetic reduction of CO_2 is known to involve the Calvin-Benson cycle. Mechanisms to account for the reaction have been proposed but it became clear that final judgment awaited separation of the algal cell into organelles in order to investigate the role of each cellular component. We have studied the oxyhydrogen reaction in the presence of radioactive CO_2 in the isolated, intact chloroplast of C. reinhardtii to determine whether this organelle is capable of assimilating CO_2 coupled to the aerobic oxidation of hydrogen or whether extra chloroplastic support is required.

The endogenous rate of CO_2 uptake was increased 3- to 4-fold by externally added ATP and additionally when combined with glucose, ribose-5-phosphate, and glycerate-3-phosphate. The rate was diminished 50 to 75%, respectively, when H_2 was replaced by N_2 or by air. Decrease of CO_2 uptake by DL-glyceraldehyde was taken for evidence that the regenerative phase and complete Calvin-Benson cycle were needed. Diminution of CO_2 uptake by rotenone, antimycin A, and DBMIB was attributed to an inhibition of the oxyhydrogen (H_2 plus O_2) reaction. That CO_2 fixation was abetted by externally added ATP indicates potential involvement of mitochondrial respiration.

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