Photosynthesis and Carbon Metabolism in a Chloroplast Preparation from *Acetabularia*

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With 6 Figures

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Summary

Preparations of chloroplasts from *Acetabularia* carry out photosynthetic activity *in vitro* that is indistinguishable in rate and products from their activity in intact cells. The activity of the preparation is stable for hours. In the isolated chloroplasts, the bulk of carbon fixation occurs through the Calvin cycle; however some β carboxylation occurs. Glycolate and glycerate are rapidly turned over suggesting that they are participating in synthetic pathways. The most obvious end product of carbon fixation is sucrose, but some starch is formed in the chloroplasts. In intact cells the carbon flows into fructans which are formed in the cytoplasm. The evidence available suggests that contamination plays at most a very small role in the activities of the isolate.

In the isolate, there is a rapid accumulation of insoluble carbon, and much of it is protein. Hydrolysis of the protein reveals that all the amino acids are derived from photosynthetic products. Work in progress has also demonstrated the biosynthesis of the plastid pigments, lipids and nucleic acids from $^{14}$CO$_2$. Thus the chloroplasts are active in biosynthesis.

The properties of the isolate suggest that the chloroplast is a tight cytoplasmic compartment. Only a limited number of compounds are exchanged with cytoplasmic pools, and control mechanisms may involve the transport of photosynthetic products.

A preliminary study of chloroplasts isolated from enucleate cells has revealed a diminished rate of carbon fixation. A tentative conclusion is reached that the deficit is perhaps in the transport of HCO$_3^-$ across the chloroplast membrane.

1. Introduction

The chloroplasts of *Acetabularia* are small, unspecialized algal chloroplasts. They possess thylacoid membranes, one to several starch grains, a nucleoid...
containing DNA, and occasional other unidentified granules. These chloroplasts have no proplastid stages and are green throughout the life cycle of the cell; although some may differentiate into amyloplasts. During the growth phase of the cell, the chloroplasts are an exponentially growing population that simultaneously carries out replication and photosynthesis. The cell in which they reside is easily disrupted without damage to the organelles. These relatively undistinguished features result in an exceptionally useful chloroplast for experimental purposes. Chloroplast preparations are readily obtained from *Acetabularia* and they have an unmatched ability to carry out both photosynthesis and a variety of biosyntheses for hours *in vitro* at normal rates. Work from our laboratories concerning the utilization of CO₂ and its fate in these chloroplasts will be reviewed.

There are some problems with the *Acetabularia* chloroplast preparations, however. The cell cultures tend to be contaminated by a variety of marine autotrophs; although techniques have been developed in several laboratories to eliminate these (GIBOR and IZAWA 1963, BERGER 1967, and SHEPHARD 1970 a). A potentially more serious problem is related to the geometry of the cell. Its cytoplasm exists as an extensive, thin sheet between the cell wall and the vacuole. When the cell is disrupted, this cytoplasmic layer fragments into droplets containing chloroplasts plus other cytoplasmic elements surrounded by a membrane—probably derived from the tonoplast membrane which originally surrounded the vacuole. Many such droplets, which are in reality small cytoplasts containing undisturbed cytoplasm and organelles, will survive subsequent manipulations unless these are carefully executed and monitored. We have developed a technique for shearing the crude isolate by passage through 5 or 8 μm straight pores etched through polycarbonate membranes (Nuclepore filters). Chloroplast diameter in the isolation media is approximately 6 μm; thus the clearance is minimal. Shearing is followed by centrifugation steps carefully designed to eliminate particles both heavier and lighter than the chloroplasts. Chloroplasts prepared by this technique have been used for many of the studies to be reported here. Evidence will be presented that if a membrane and some cytoplasmic elements remain after this procedure, they contribute little or nothing, besides stability perhaps, to the observed properties and activities of the chloroplast preparation.

2. Methods

The cells used for these experiments were stocks of *Acetabularia mediterranea* which were maintained in continuous laboratory culture under controlled conditions in a synthetic medium. The cultures were free from other photosynthetic species, molds and yeasts; many of them, however, had a low level of contamination by *Pseudomonas* spp. which was monitored by plate counts and kept very low. These bacteria can be eliminated from chloroplast preparations (see below). The methods for maintaining these cultures have been described in detail (SHEPHARD 1970 a).