Relative Requirements for Magnesium of Protein and Chlorophyll Synthesis in *Euglena gracilis*  

Received for publication May 17, 1977 and in revised form December 12, 1977

RAYMOND E. ZIELINSKI AND CARL A. PRICE  
Waksman Institute of Microbiology, Rutgers University, P.O. Box 759, Piscataway, New Jersey 08854

ABSTRACT

The relationships among Mg, growth, chlorophyll synthesis, and cytoplasmic polysome content were studied in *Euglena gracilis* grown in different levels of the metal. At all levels of magnesium from 20 to 1,600 μmolar, both protein and chlorophyll are formed with exponential kinetics. The apparent rates of synthesis and final yields of both components are greater at higher levels of Mg, but the rate of chlorophyll synthesis always exceeds the rate of protein formation; i.e. the most severely deficient cells contain proportionally more chlorophyll than the sufficient cells. Cytoplasmic polysomes isolated from Mg-deficient *Euglena* are indistinguishable from those isolated from control cells. We conclude that decreased rates of protein synthesis occur prior to and possibly are causal to decreased rates of chlorophyll synthesis, but that the mechanism of this inhibition remains unclear.

Magnesium is among the essential macronutrients of plants (14), but the biochemical basis for this requirement has not been clearly established. Indeed, the problem lies in choosing from among many possible bases for a Mg requirement. The need for Mg has been variously ascribed to its occurrence in Chl and its action as a cofactor for a variety of enzymes, notably those of phosphate transfer (17). Specific requirements for Mg have also been reported for the development of chloroplasts (7, 16) and mitochondria (11). Since the complexes of Mg with enzymes have relatively low affinities and the metal is easily translocatable throughout the plant, compositional studies can be difficult to interpret. We have employed progressive Mg deficiency in *Euglena gracilis* (12) to inquire into the relative priorities between the synthesis of protein and Chl in the cell. Specifically, we asked if a failure in the production of Chl might precede a decrease in the over-all rate of protein synthesis.

As our test organism we chose *Euglena gracilis* with glucose as the principal carbon source. Under photoheterotrophic conditions, chloroplast development is gratuitous and therefore can easily be separated from global protein synthesis.

MATERIALS AND METHODS

Our strain of *E. gracilis* (Klebs) is derived from the z strain of Pringsheim, but differs from it in the morphology of its thylakoids. The cells were cultured photoheterotrophically on a modified Huntner medium as described previously (5). Mg was added as the sulfate salt. Constant levels of sulfate were maintained by adding appropriate amounts of KHSO₄. In order to minimize the carry-over of Mg, transfers of algae were made from exponentially growing stock cultures containing 100 μM MgSO₄, which is one-sixteenth the normal concentration. There was zero growth without added Mg.

Growth was monitored turbidimetrically at 540 nm. Chl was estimated by a modification of Arnon's (1) method (3) and protein by the Lowry method (10). All glassware was washed by soaking in 50% (v/v) nitric acid for 2 to 3 weeks prior to use.

Polysomes were isolated from midexponential phase cultures by the method of Avadhani and Buetow (2) with the high pH modification of Davies *et al.* (4).

RESULTS AND DISCUSSION

Kinetics of Growth and Formation of Chl and protein. When photoheterotrophic *Euglena* are grown with different amounts of Mg initially present in the medium, both the rate of growth and total yield, as measured by apparent optical density, are affected (Fig. 1A). Provided that exponentially growing cells were used to inoculate the cultures, no lag phase was observed. Protein content follows a nearly identical pattern, as shown in Figure 1B. Both increase exponentially at all of the concentrations tested but with progressively lower rate constants. The total yield of protein is also progressively decreased, with the yield at 20 μM Mg lowered to about 30% of the control culture at 100 hr.

The rates of Chl accumulation are similarly exponential, with decreasing rate constants at the lower levels of Mg (Fig. 1C). The Chl doubling times, however, are always slightly less than the generation times of the respective cultures. Throughout the exponential stages of growth, the ratios of Chl to protein (Fig. 2) vary only slightly, and then without any systematic relation to the concentration of Mg.

These results contrast with similar studies employing iron deficiency in *Euglena* (5, 13). Using virtually identical conditions, Funkhouser and Price (5) found that Chl synthesis was halted at low levels of iron while protein accumulation continued at normal exponential rates. Those results were consistent with a rather strict compartmentalization of iron within the cell. In contrast, we find that growth and Chl synthesis are not separable under conditions of progressive Mg deficiency and that by inference no such compartmentalization exists for Mg.

Our data do not support a regulation of the synthesis of the Chl molecule by Mg per se. The correlation between total protein content and Chl accumulation (Fig. 2 inset) is consistent with the notion that protein synthesis controls the accumulation of Chl in Mg-deficient photoheterotrophic *Euglena*. This conclusion is further supported by evidence that protein synthesis is required for normal Chl production when etiolated plant tissues are exposed to light (6, 9). In view of this seeming dependency, it is surprising to find that the rate of Chl accumulation, while parallel to, is always greater than the rate of protein synthesis.

The decrease in protein and Chl synthesis caused by Mg deprivation is a reversible one. The addition of 1,600 μM Mg to cells

---

1 Supported in part by Grant HD-05602 and by a biomedical research support grant from the National Institutes of Health to Rutgers University.
that had grown to stationary phase in 20 μM Mg stimulated cell density and the yield of Chl to nearly normal levels (data not presented). We take this to mean that the changes observed in the cultures were attributable to the level of Mg initially present in the growth medium and not to irreversible changes in the cells.

The stationary phase is usually glucose-limited and the photoheterotrophic cells become autotrophic. During early stationary phase, Chl synthesis continues at a rate that is sharply diminished (Fig. 1C) but nonetheless more rapid than protein synthesis. The Chl to protein ratios of low Mg cultures increase more slowly, to a lesser extent, and at a later time after inoculation than with Mg-sufficient cultures. A proportional plot of Chl against protein (Fig. 2 inset) indicates, however, that on the basis of the protein accumulation, the cells grown in 20 μM Mg are slightly Chl-rich.

Polysome and RNA Levels. Since we found that Mg deficiency affects the apparent rate and extent of protein synthesis in Euglena, we wondered if the lesion could be at the level of the polysomes. We isolated polysomes from midexponential phase cultures of Euglena grown in different concentrations of Mg. The sedimentation profiles for different cultures were compared with respect to two criteria: the ratio of polysomes to total ribosomes; and the size distribution of the polysomes. Our results (not shown) indicate that polysome profiles obtained from Mg-deficient Euglena do not differ significantly from Mg-sufficient cultures. Polysomes extracted from all cultures tested were comprised about 10% of the total cellular RNA and about 50% of the total ribosomes, with pentamers or hexamers the most abundant.

Although there is much evidence demonstrating the need for high levels of Mg by several components of the protein-synthesizing machinery (2, 8, 15) and our results point to a decline in protein synthesis during progressive Mg deficiency, the results obtained from the isolation of polysomes from Euglena are inconclusive. We note that our methods recovered only a small fraction of the total ribosomes of the cell and that Mg itself is required to isolate polysomes; so that substantial differences in the polysome content in vivo might have been missed.

Although our experiments were performed with photoheterotrophically grown organisms, we see no reason to expect that these priorities for Mg would differ within an autotrophic organism. Apart from the advantages of working with a microorganism under controlled conditions, photoheterotrophic Euglena provides a better test for studying the role of Mg than would an obligately autotrophic higher plant. If the production of Chl were essential to the maintenance of normal growth rates, a tight coupling between protein and Chl accumulation, such as we observed, could well have been interpreted to mean that protein synthesis requires Chl formation rather than the other way around.

LITERATURE CITED