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Endogenous Dark Respiration of the Blue-Green Alga, *Plectonema boryanum*

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Endogenous dark respiration in the blue-green alga *Plectonema boryanum* is markedly affected by preincubation in the light: it can be increased from a basal rate of 5 nmoles of O_2 to 55 nmoles of O_2 per mg of cell protein per min after exposure of the cells to light for 8 to 10 hr. Under conditions of enhanced dark respiration, cyanophage multiplication in the dark increases drastically and approaches the cyanophage yields obtained in photosynthesizing *Plectonema* cells. This implies that the biosynthetic capabilities of the algal cells, at least with respect to viral synthesis, can be similar in the dark to those in the light. The enhanced endogenous respiration rate was found to be dependent on photoassimilation of CO_2 and on protein synthesis. The implications of these findings with respect to obligate photoautotrophic metabolism in blue-green algae are discussed.

Studies of the blue-green algae in recent years have concentrated mainly on their structure (13) and photosynthesis (9). These investigations showed that the blue-green algae are procaryons having a eucaryotic type of photosynthesis. This condition prompted the study of the still enigmatic respiratory mechanism of these algae and its relation to their obligate photoautotrophic metabolism. In addition, such an investigation might shed light on the evolution of the two main energy-yielding mechanisms—photosynthesis and respiration. In this connection, it is interesting that both in photosynthetic bacteria and in blue-green algae the photosynthetic and respiratory systems have been found located together in the photosynthetic lamellae and seem to be functionally linked (4, 9, 22).

Dark endogenous respiration has been demonstrated in several blue-green algae (1, 12, 24). A number of authors (1, 12) have shown that this endogenous respiration shows a limited (if any) response to the level of metabolites in the medium. Accordingly, until now, very few blue-green algae have been demonstrated to grow in the dark on exogenous metabolites (3, 11, 14, 23). The dark endogenous respiration in blue-green algae might be related to an endogenous photosynthetic substrate since starvation in the dark brought about a drastic decrease in the oxygen uptake (12).

Further studies aimed at resolving the nature of the enzymatic chain and the metabolic role of the respiratory system in blue-green algae led to controversial conclusions (10, 15, 20). It seemed advisable to us first to elucidate the general relationship between dark endogenous respiration and photosynthetic metabolism in blue-green algal cells as a basis for a detailed study of individual reactions.

This paper describes the relations of oxygen uptake to photoassimilation of CO_2, protein synthesis, and cyanophage multiplication in *Plectonema boryanum* cells incubated under various conditions of light and dark.

MATERIALS AND METHODS

Alga and cyanophage: growth and quantitation. *P. boryanum* 594 (Gomot) from the University of Indiana culture collection (Bloomington, Ind.) and cyanophage LPP1-G, isolated and characterized by Padan et al. (16) were used.

Standard growth conditions of the alga. The growth medium used in this study was a modified CHU no. 10 mineral solution described by Safferman and Morris (19). The alga was grown in stationary cultures at 24 to 26 C and continuously illuminated by 10^4 ergs per cm^2 per sec of incident white light. Quantitation (number, cell protein) of *Plectonema* cells and the cyanophage have been described (5, 16). The determination of the intracellular growth curve of the cyanophage was described previously (17).

Experimental incubation conditions. *Plectonema* cells, harvested in the logarithmic phase of growth, were incubated in light (incident intensity, 4.5×10^4 ergs per cm^2 per sec) or in the dark in the bicarbonate medium as described (5) under identical conditions of agitation and temperature (26 C). Exclusion of CO_2 from the standard experimental system was achieved as de-

scribed (17), but the cells were suspended in the standard medium lacking carbonate, to which 0.01 M glycylglycine was added and titrated to *p*H 9 with NaOH.

Determination of O_2 consumption. Washed algal cells were concentrated to 0.3 to 0.4 mg of protein/ml in the bicarbonate medium. The O_2 uptake was determined in 3-ml cell suspensions darkened with aluminium foil, with a Clark-type oxygen electrode (Yellow Springs Instrument Co., Yellow Spring, Ohio).

Determination of photoassimilation of CO_2 and protein synthesis in the algal cells. CO_2 photoassimilation by *Plectonema* cells was determined as previously described (5). For the measurement of protein synthesis, leucine and ^{14}C-leucine (Radiochemical Centre, Amersham, England) were added to the incubation mixture to give a final concentration of 1 μmole/ml and specific activity of 1 μCi/μmole. At different time intervals, samples were removed and incubated for 5 min at 4 C with trichloroacetic acid (final concentration 10%). The incubation mixture was then drawn through membrane filters (HA, Millipore Corp., Bedford, Mass.) of 0.45 μm average pore size. The precipitate retained on the filter was washed with 100 ml of 10% cold trichloroacetic acid. Samples were counted in a gas-flow, end-window planchet counter (model D-47, Nuclear Chicago Corp., Des Plaines, Ill,). Self-absorption was negligible, and the counts were corrected for background radiation. Counting efficiency was approximately 15%. Since 90 to 95% of the label was extracted from the cold trichloroacetic acid precipitate (dialyzed against and resuspended in 0.05 M potassium phosphate buffer, *p*H 7.3) by incubation at 37 C for an hour with 1 mg of Pronase (Calbiochem)/ml, it was concluded that ^{14}C-leucine incorporation represents protein synthesis.

Inhibitors. 3-(3,4-Dichlorophenyl)-),1-dimethylurea (DCMU) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were kindly supplied by E. I. Du Pont de Nemours & Co., Inc. Erythromycin ethyl carbonate was purchased from RAFA laboratories, Jerusalem, Israel, and chloramphenicol (CAP) from ABIC, Ramat Gan, Israel.

RESULTS

Endogenous respiration in light-incubated Plectonema cells. The rate of dark endogenous respiration of logarithmic-phase *Plectonema* cells was found to be 15 to 20 nmoles of O_2 per mg of cell protein per min (Fig. 1). When such cells were incubated under dark conditions for an hour prior to the determination of oxygen uptake, the endogenous respiration rate decreased to 5 to 10 nmoles of O_2 per mg of cell protein per min (Fig. 1). This minimal rate of endogenous respiration remained constant during at least 6 days of dark incubation.

When *Plectonema* cells were incubated in the experimental incubation conditions in the light (conditions which are optimal for CO_2 photoassimilation) prior to the oxygen uptake determination, a marked increase in the dark endogenous respiration was observed (Fig. 1). The increment in the endogenous respiration rate is directly related to the duration of the light incubation up to a maximal rate (45 to 55 nmoles of O_2 per mg of cell protein per min) reached after 8 to 10 hr of incubation in the light.

To investigate the stability of the endogenous respiration rate at its various levels, different *Plectonema* cell suspensions were first incubated in the experimental light conditions for various periods and then incubated in the dark for different times. Rates of oxygen uptake in these various incubation systems are presented in Table 1. Although the endogenous respiration rates after dark incubation are variable, there appears to be a general tendency towards a decreasing rate with increased time of dark incubation.

Cyanophage multiplication. We have found that logarithmic-phase *Plectonema* cells infected with LPP1-G cyanophage can support some measure of cyanophage synthesis when incubated in the dark (17). It was therefore interesting to determine whether cyanophage multiplication is affected in those *Plectonema* cells having enhanced dark endogenous respiration rates. Algal cells were incubated in light conditions for various times before cyanophage infection, and the intracellular growth curves of the cyanophage in the dark were determined (Fig. 2). The growth rate and yield of the cyanophage are related to the length of the light incubation period. The maximal dark yield (which is 50% of the light yield) is obtained after 10 to 12 hr of incubation in the light; this is the time of light incubation necessary for maximum dark endogenous respiration (*see* Fig. 1). Furthermore, the cyanophage multiplication in the dark is inhibited by CCCP, an uncoupler of oxidative phosphorylation (6). The similar residual cyanophage yields obtained in the presence of CCCP and after a long dark incubation may be due to anaerobic metabolism.

The results imply that the endogenous respiration enhanced by light incubation can support metabolic processes involved in cyanophage synthesis at half, at least, of the efficiency obtained during photosynthesis of the host cells.

Effect of photosynthetic reactions on dark endogenous respiration. Since light incubation induced an increased dark endogenous oxygen uptake, it was interesting to determine whether photosynthetic reactions are involved in this effect. Thus, different photosynthetic reactions were excluded or inhibited during the experimental light incubation.

In blue-green algae, CCCP is both an uncoupler of photophosphorylation (2) and inhibitor of photosynthetic electron transport (21). The addition of CCCP at the beginning of the experi-

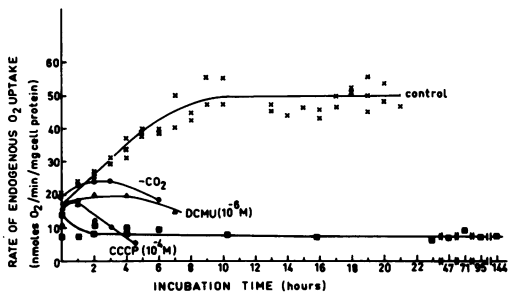

FIG. 1. *Dark endogenous respiration in Plectonema boryanum cells incubated under various conditions. Logarithmic-phase Plectonema cells were incubated for different times in the light* (×) *and in the presence of the inhibitors of photosynthesis, DCMU* (▲) *or CCCP* (○), *and in the absence of* CO_2 (●) *or in the dark* (■). *The endogenous dark respiration rate was determined immediately after the given times of incubation in the light.*

TABLE 1. *Stability of the dark endogenous respiration of Plectonema cells at its various levels*[a]

Dark incubation (hr)	Rate of endogenous dark respiration[b] after light preincubation for						
	0 hr	2 hr	3 hr	4 hr	6 hr	14 hr	15 hr
0	15.9	26.6	35.6	40.3	39.2	44.9	48.5
1	11.3	25.2	29	28.8	26	39.2	36.5
2	14.1	14.3	21.5	23	21.6	39.2	26.5
3		17.4	25.7	34.5	26	39.2	27.4
4	12.9	13.3	24.0	28.8	19.5		29.7
5					19.5	39.2	23.4
6	13.4						17.9
7							21.7
8							24.2

[a] Logarithmic-phase *Plectonema* cells were preincubated in the experimental light condition for various periods and then incubated in the dark for different times. The endogenous dark respiration rate was determined in these various incubation systems. The results represent averages of at least five experiments.

[b] Expressed as nanomoles of O_2 per milligram of cell protein per minute.

mental light incubation of algal cells inhibited the increment in oxygen uptake (Fig. 1). It can thus be concluded that the enhancement of dark endogenous respiration is related to products of the photosynthesis and not to some other effect of light. The fast rate of decay of dark endogenous respiration in the presence of CCCP may be due to damage of the cells due to lack of energy.

In blue-green algae, DCMU inhibits the algal photosystem II, in both in vivo (17) and in vitro (21) conditions, thus blocking photosynthetic reduced nicotinamide adenine dinucleotide phosphate (NADPH) production and CO_2 photoassimilation, whereas adenosine triphosphate (ATP)

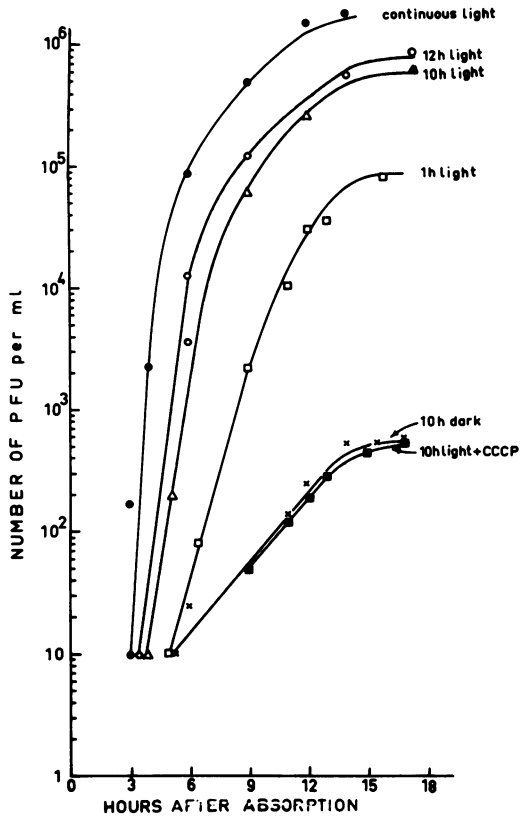

FIG. 2. *Intracellular growth curve in the dark of LPP1-G in Plectonema cells incubated under various conditions before the beginning of the latent period. Logarithmic-phase Plectonema cells were incubated in the light for different periods, in the presence of* 10^{-4} *M CCCP or in the dark before infection with an input multiplicity of 0.1 plaque-forming cyanophage units/algal trichome. Unadsorbed phages were removed and light was excluded in the light-incubated systems. The experimental system incubated in continuous light served as a control.*

continues to be synthesized at a slower rate by cyclic photophosphorylation. Accordingly, inhibition of the photoassimilation of CO_2 in *Plectonema* incubated with DCMU is observed within 60 min (Fig. 3). In the experimental conditions shown in Fig. 1, DCMU inhibits the increment in dark endogenous respiration within an hour. Thus, it is evident that the products of the noncyclic electron flow are needed to produce the increase in the rate of endogenous respiration. The slower decay of the dark endogenous respiration rate observed after experimental light incubation in the presence of DCMU, as compared with dark incubation, may be explained by the fact that cyclic photophosphorylation still operates in the presence of DCMU.

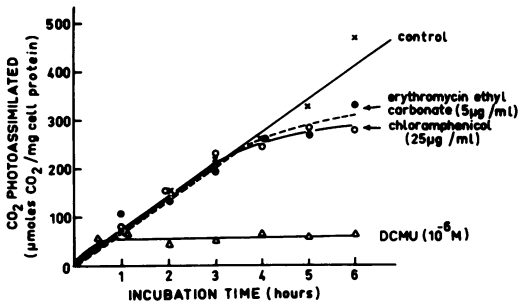

FIG. 3. *CO_2 photoassimilation by Plectonema boryanum in the presence of various inhibitors. Logarithmic-phase Plectonema cells were incubated in the light with inhibitors or without (control). The photoassimilated CO_2 was determined at different times of incubation.*

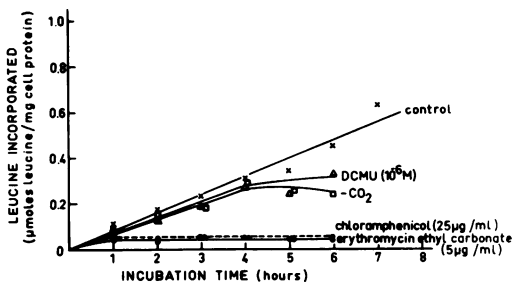

FIG. 4. *Leucine incorporation by Plectonema boryanum cells during inhibition of metabolic processes. Logarithmic-phase Plectonema cells were incubated in the light in the presence of various inhibitors or in the absence of CO_2. The leucine incorporated was determined at different times.*

The main reaction supported by the products of the noncyclic electron flow is the photoassimilation of CO_2. We have previously shown (17) that it is possible to exclude CO_2 photoassimilation while sufficient NADPH and ATP are supplied for cyanophage synthesis. Figure 1 shows that incubation of *Plectonema* cells in the light without CO_2 did not increase the rate of the dark endogenous respiration.

To test whether DCMU or absence of CO_2 have an effect independent of photoassimilation on dark endogenous respiration, the dark O_2 uptake of light-incubated *Plectonema* cells was determined in the presence of DCMU or in the absence of CO_2. Under these conditions, no changes in the increased rate of O_2 uptake were observed. It is concluded that products of the photoassimilation of CO_2 may be required for the enhanced dark endogenous respiration.

Protein synthesis. The absence of CO_2 photoassimilation may affect dark respiration through interference with protein synthesis. Therefore, this latter parameter was determined in the presence of DCMU or in the absence of CO_2. Protein synthesis was inhibited by lack of CO_2 photoassimilation only after 4 hr of incubation (Fig. 4). Because of this time lapse, it is unlikely that inhibition of protein synthesis is responsible for the immediate effect on the dark respiration increase caused by inhibition of photoassimilation (Fig. 1).

Nevertheless, it was interesting to determine whether the increased endogenous dark respiration itself has a requirement for protein synthesis. When *Plectonema* cells were exposed to CAP during the light incubation period, incorporation of ^{14}C-leucine into the algal protein was inhibited within an hour after addition (Fig. 4), and the enhanced dark endogenous respiration was suppressed (Fig. 5). To test whether CAP has an effect in addition to protein synthesis inhibition in the light, dark O_2 uptake of light-incubated *Plectonema* cells was determined in the presence of CAP. Fully enhanced dark respiration was, nevertheless, observed.

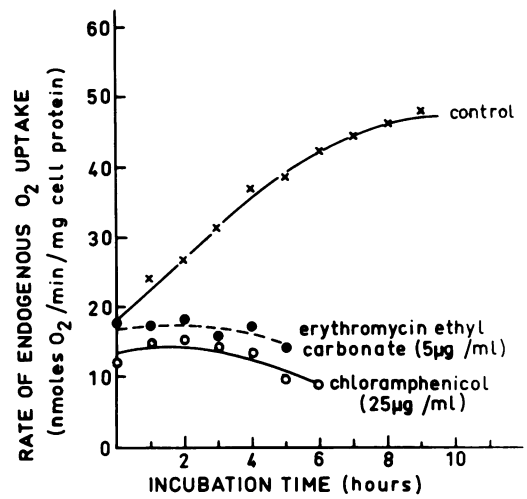

FIG. 5. *Endogenous dark respiration of Plectonema boryanum cells in the presence of protein synthesis inhibitors. Logarithmic-phase Plectonema cells were incubated in the light in the presence of chloramphenicol* (○) *and erythromycin ethyl carbonate* (●). *Endogenous dark respiration rate was determined after the given periods of light incubation.*

When CO_2 photoassimilation was determined in the presence of CAP (Fig. 3), photoassimilation continued at the control rate for at least 3 hr. Thus, the effect of CAP on CO_2 photoassimilation cannot be related directly to its effect on dark endogenous respiration.

To exclude the possibility that CAP is unique in its effect on respiration, another inhibitor, erythromycin-ethyl carbonate, was tested for its effects on protein synthesis (Fig. 4), CO_2 photoassimilation (Fig. 3), and oxygen uptake (Fig. 5); it was found to have an effect similar to CAP. It appears that protein synthesis in the light is re-

quired for the increased endogenous dark respiration and is independent of the requirement for CO_2 photoassimilation.

DISCUSSION

The results show that the dark endogenous respiration of the blue-green alga *P. boryanum* can be varied. The dark respiration rate of *Plectonema* cells grown under standard conditions and harvested at the logarithmic phase of growth is 15 to 20 nmoles of O_2 per mg of cell protein per min. When these cells are incubated in the dark, the dark endogenous respiration rate decreases to 5 nmoles of O_2 per mg of cell protein per min; on the other hand, such cells incubated in conditions of optimal photoassimilation (maximal light and CO_2) have a dark endogenous respiration rate of 45 to 55 nmoles of O_2 per mg of cell protein per min.

The biochemical capabilities of the algal cells in the dark are drastically changed by the light incubation, at least with respect to viral synthesis. This is the implication of the relative efficiency of cyanophage multiplication in the dark that is dependent on the dark endogenous respiration enhanced by light incubation (from less than 1 to 50% of the cyanophage yield obtained in photosynthesizing cells in continuous light). In view of this, it is surprising that so few blue-green algae have been found to grow in the dark on exogenous substrates, especially considering that such substrates penetrate the cells and enter its metabolic pathways (7, 9, 18) and that oxidative phosphorylation was demonstrated in these algae (15).

The metabolical reactions found to influence the dark endogenous respiration of *Plectonema* are the photoassimilation of CO_2 and protein synthesis. The need for CO_2 photoassimilation is readily explained by the need for a substrate for oxidation. The requirement for protein synthesis may imply that a protein factor or factors of the respiratory chain have to be synthesized in the light before an increment in the dark respiration is obtained. This may indicate that there exists a control mechanism relating the level of respiration to the level of photosynthetic products. The algal dark respiratory system may be connected with photosynthesis through a particular substrate or substrates serving for oxidation and triggering the synthesis of the unstable algal respiratory pathway. In this case, it would be very difficult to maintain dark endogenous respiration at the higher level for long periods and, consequently, a high aerobic growth rate in the dark. This is supported by the immediate, though slow, decay of the enhanced dark respiration rate, even from its highest level. In addition, the demonstrated growth in the dark of the blue-green algae was extremely slow (3, 11, 14, 23).

The minimal rate of the endogenous dark respiration observed here corresponds to the values of the dark respiration noted previously for other blue-green algae (1, 12, 24). However, nothing like the maximal rate of dark respiration has been demonstrated before for a blue-green alga. We suggest that the previous tests of endogenous respiration in blue-green algae were made in cells maintained at or near the basal state, which explains the stability claimed for the dark respiration of blue-green algae (1, 24).

Further, as already mentioned, studies of in vitro dark respiration of blue-green algae have given controversial results. Horton (10) found that particles isolated from blue-green algae possess a cyanide-sensitive NADH oxidase, whereas Smith et al. (20) reported the absence of such enzymes and suggested that this deficiency represents a major biochemical basis for obligate photoautotrophy in these organisms. Further, the latter investigators speculated that the process of oxidative phosphorylation may not exist in blue-green algae and that all ATP is generated in the dark by substrate-level phosphorylation. According to them, endogenous respiration seems to have little metabolic significance in these algae. On the other hand, Leach and Carr (15), directly, and Biggins (1), indirectly, demonstrated the coupling of oxidative phosphorylation to NADPH oxidation by blue-green algal preparations. Hence, the latter authors suggested that the oxidation of endogenous substrates by these organisms is mediated by some NADP-linked enzyme and that this electron transfer is coupled to phosphorylation. These contradictory results might be explained in the light of the finding that dark respiration is so intimately related to the physiological state of the cell. Thus, when comparing dark respiration of different blue green algae, in both in vivo and in vitro preparations, the physiological state of the cells must be considered carefully.

Hoch et al. (8) found that two mechanisms of oxygen uptake operate in the blue-green alga *Anacystis nidulans* during illumination, identifying one as endogenous dark respiration and the other as light-dependent oxygen uptake. Our findings indicate that the relationship between these two kinds of respiration should be investigated further.

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