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METABOLIC ASPECTS OF LPP CYANOPHAGE REPLICATION IN THE CYANOBACTERIUM *PLECTONEMA BORYANUM*

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SUMMARY

Cyanophage LPP1G is reproduced at the same yield in heterotrophic conditions (dark, glucose) as in photoautotrophic conditions; aerobiosis is required for dark cyanophage replication. Exogenous glucose is not required for the cyanophage replication in the dark in heterotrophically grown cells. In photoautotrophically grown cells, the maximum burst size in dark and glucose is delayed for a period corresponding to glucose uptake induction. Cyanophage LPP2SPI replication occurs in conditions where only Photosystem I operates. Of photosynthesis parameters tested, only CO_2 photoassimilation is affected during cyanophage LPP1G infection under photoautotrophic conditions.

INTRODUCTION

Cyanobacteria are procaryotic organisms capable of oxygenic photosynthesis, and thus occupy a special position in the biological world. This has been the major stimulus for the study of the interaction between these organisms and their viruses, the cyanophages. Although the dominant metabolic pattern in cyanobacteria is photoautotrophism, some forms can grow in the dark using carbohydrates as carbon and energy sources. A study of the interaction of cyanophage development and the cyanobacterial metabolism can contribute to an understanding of eucaryotic photosynthesis under viral infection and of host autotrophic and heterotrophic metabolic potentials under different environmental conditions.

The cyanobacterium *Plectonema boryanum* and its cyanophages LPP1G and LPP2SPI offer a suitable system for investigating the metabolic potential of cyanobacteria. *Plectonema* is capable of operating alternate modes of metabolism: photoautotrophic metabolism and heterotrophic metabolism induced by preincubation in optimum photosynthetic conditions [1] or by incubation with glucose in the dark or in light in the presence of (dichlorophenyl)-dimethylurea [2].

Several aspects of the interaction between cyanophage LPP1G reproduction and the photoautotrophic metabolism of *Plectonema* have already been described. It was found that multiplication of cyanophage LPP1G in photoautotrophic *Plectonema*

depends on photophosphorylation during the eclipse period which can be mediated by cyclic photophosphorylation [3]. It was also shown that infection with cyanophage LPP1G is followed by severe damage to the host photosynthetic system expressed by folding of the photosynthetic lamella [4–6] and complete inhibition of CO_2 photoassimilation [7, 8].

In the present study we have investigated the capacity of *Plectonema* cells to support cyanophage replication in heterotrophic conditions, and have further analyzed the behavior of the photosynthetic capacity during the cyanophage developmental cycle.

MATERIALS AND METHODS

Growth of cyanobacteria and cyanophages

P. boryanum 594 (Gomot) from the Indiana University Culture Collection (Bloomington, Ind.) served as host. Photoautotrophic growth conditions for *P. boryanum* were as described by Ginzburg et al. [7]. This medium was modified for heterotrophic growth in the dark by addition of 30 mM glucose. All heterotrophic growth experiments were conducted with a streptomycin-resistant mutant of *P. boryanum* capable of growing in the presence of 50 μg streptomycin/ml. Cyanophage strains used were LPP1G isolated and characterized by Padan et al. [9] and LPP2SPI obtained from R. S. Safferman (Virology Section, EPA, FWQA, Cincinnati, Ohio). Methods for concentration, purification and enumeration of cyanophages have been described [7, 9]. Burst size (plaque forming units/infected cell) and burst time were obtained from intracellular growth curves [3]. In one experiment (see Table II), light was provided by 1000 W tungsten-halogen photographic lamp (Atlas, Pl/15, U.K.). The light was filtered through Baird Atomic sharp cut-off interference filter blocked to infinity, peaking at 700 nm (25 nm half band width), prefiltered by Corning H. R. 2-60 filter. The intensity of the actinic light was equal to $1.2 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}.

Photosynthetic parameters

CO_2 photoassimilation rate was determined by following continuous incorporation of radio-labelled bicarbonate in *Plectonema* cells as described [7]. This rate was also determined by a pulse technique in which $NaH^{14}CO_3$ was added (to obtain a final specific activity of 0.5 Ci/mol) to samples of infected *Plectonema* cells prepared as above in growth medium containing 20 mM $NaHCO_3$; these samples were incubated for 10 min in light, collected on 0.45 μm (average pore size) Millipore filter and washed with 10 % cold trichloroacetic acid. Radioactivity was counted in a gas flow counter.

Ribulose-1,5-diphosphate carboxylase activity was detected according to Wishnick and Lane [10] in *Pectonema* cell extracts prepared by mechanical breakage of cells [3] suspended in 10 mM Tris buffer (pH 7.6).

Chlorophyll *a* and phycocyanin concentrations were measured in the *Plectonema* cell extract prepared in 0.1 M phosphate buffer (pH 7). Phycocyanin was measured in the supernatant (12000 $\times g$, 10 min, 4 °C) by its absorbance at 620 nm [11]. Chlorophyll *a* was extracted from the pellet with 80 % acetone, and its concentration was measured by absorbance at 663 nm [12].

O_2 evolution in intact cells was measured with a Clark-type oxygen electrode

as described for O_2 consumption [1]. The reaction was started by turning on a tungsten lamp (500 W, General Electric); the incident light intensity ($5 \cdot 10^4$ ergs $\cdot$ $cm^{-2} \cdot s^{-1}$) was saturating for O_2 evolution which was linear for at least 5 min at 26 °C.

For measuring photophosphorylation and O_2 evolution in cell-free preparations, spheroplasts of *Plectonema* cells were prepared as described by Biggins [13] except that the lysozyme treatment was carried out in the dark for 40 min [14]. Spheroplasts were washed and suspended in cold 0.04 M Tricine/maleate buffer (pH 7.5) which contained 0.5 M mannitol. The reaction mixture for measuring light reactions in lysed spheroplasts contained (per 3 ml) 80 μmol Tricine/maleate buffer (pH 7.5), 60 μmol $MgCl_2$, 10 μmol K_2HPO_4 (pH 7.8) containing ^{32}P ($3 \cdot 10^6$ cpm), 6 μmol NADP and 10 μmol ADP, 0.2 mg ferredoxin, and 0.1 ml diaphorase (saturating amount) prepared from Swiss chard (obtained from E. Tel-Or, Weizmann Institute). A suspension of spheroplasts determined to contain 30–50 μg chlorophyll was added to the reaction mixture. O_2 evolution during illumination for 2 min was determined as in intact cells, and was terminated with 3 % trichloroacetic acid. Photophosphorylation was measured in the same sample according to labelled ATP [15]. Cyclic photophosphorylation was followed by adding 0.1 μmol phenazine methosulfate to a similar reaction mixture.

Total ATP in infected *Plectonema* cells was determine by filtering 10^8 cells through a 3-μm (average pore size) Millipore filter under low pressure, boiling the filters for 5 min in 5 ml 0.02 M Tris buffer (pH 7.75), cooling and centrifuging ($12000 \times g$, 10 min, 4° C). ATP was detected in the supernatant using the firefly luciferin-luciferase method [16].

RESULTS

Cyanophage replication in P. boryanum in the dark

The capacity of cyanophage LPP1G to reproduce in *Plectonema* cells, grown previously either in photoautotrophic or in heterotrophic conditions, is shown in Table I. Both types of cells produced cyanophage LPP1G in aerobic dark conditions in the presence of glucose at the same efficiency as phage production in photoautotrophically grown cells in the light. While the burst time in the dark in cells previously grown heterotrophically was identical to the burst time in photoautotrophically grown cells in the light, it took about 17 h more to produce a burst in dark in cells grown photoautotrophically. The relatively low cyanophage burst size in the dark from photoautotrophically grown cells was enhanced 10-fold in the presence of glucose during the infection cycle, while the viral dark burst size in heterotrophically grown cells did not depend on the presence of external glucose. LPP1G cyanophage development in the dark was arrested in anaerobic conditions in both kinds of growth conditions.

Capacity of Photosystem I to support cyanophage LPP2SPI replication

We observed that cyclic photophosphorylation operates in vivo in *Plectonema* infected with cyanophage LPP1G [3]. To investigate whether this is also the case for *Plectonema* infected with a serologically different cyanophage we measured the replication of LPP2SPI cyanophage [17] in *Plectonema* cells illuminated with 700 nm

TABLE I

REPLICATION OF CYANOPHAGE LPPIG IN *PLECTONEMA BORYANUM*

Logarithmic-phase *Plectonema* cells, grown either photoautotrophically or heterotrophically in the dark, were washed and suspended in the growth medium without glucose, and infected with LPP1G cyanophage at an input multiplicity of 0.006 plaque-forming units/cell. After the adsorption and removal of unadsorbed cyanophages, the infected cells were incubated in conditions indicated in the table. Glucose was added to a concentration of 30 mM and anaerobiosis was performed by N_2 flushing. Phage burst size was obtained from intracellular growth curve.

Conditions during infection cycle		LPP1G burst size (PFU/infected cell) and burst time (hours in brackets)	
		Photoautotropic grown cells	Heterotrophic grown cells
Dark			
Aerobic	+glucose	300 [30]	300 [13]
	−glucose	30 [13]	300 [13]
Dark			
Anaerobic	+glucose	–	5 [13]
	−glucose	–	1 [13]
Light			
Aerobic	−glucose	300 [13]	–

wavelength light in the presence of (dichlorophenyl)-dimethylurea. Photosystem II activity is repressed by (dichlorophenyl)-dimethylurea, and only Photosystem I is activated by the actinic light. Under the given experimental conditions (Table II) the cyanophage LPP2SPI burst size in the dark was negligible whereas illumination with 700 nm wavelength light was as efficient as white light (both in the presence of (dichlorophenyl)-dimethylurea) for LPP2SPI replication.

TABLE II

PHOTOSYSTEM I-DRIVEN VIRAL MULTIPLICATION

Photoautotrophically grown *Plectonema* cells in the logarithmic growth phase were incubated in the dark for 16 h. Cells were then washed, resuspended in enriched bicarbonate medium and infected with cyanophage LPP2SPI at an input multiplicity of 0.1 plaque-forming units/cell. After 1 h adsorption in the dark (26 °C) infected cells were washed and incubated for 1.5 h in white tungsten light or 700 nm wavelength light and 10^{-6} M (dichlorophenyl)-dimethylurea. Intracellular growth was then determined in the dark at 26 °C.

Incubation conditions	Cyanophage LPP2SPI burst size (plaque-forming units/infected cell)
Red light + (dichlorophenyl)-dimethylurea	130
White light + (dichlorophenyl)-dimethylurea	130
Dark	5

Photosynthetic capacity in P. boryanum infected with cyanophage LPP1G

Different photosynthetic parameters were studied in *Plectonema* infected with LPP1G cyanophage and the values obtained at the end of the latent period (6 h after adsorption) are shown in Table III.

CO_2 photoassimilation in intact cells was the only one of these parameters drastically inhibited during LPP1G cyanophage infection. The kinetics of inhibition of CO_2 photoassimilation are demonstrated in Fig. 1. Using pulses of labelled $NaHCO_3$ (Fig. 1B), the capacity of infected cells to photoassimilate CO_2 was shown to be damaged between the second and the third hour after adsorption, the time at which inhibition of continuous incorporation of CO_2 became evident (Fig. 1A). From this time, the rate of CO_2 fixation decreased until the fifth hour after adsorption when infected cells no longer fix CO_2.

While CO_2 photoassimilation in vivo was inhibited during infection with cyanophage LPP1G, the in vitro activity of ribulose-1,5-diphosphate carboxylase which catalyzes the carboxylation reaction was unaffected. Chlorophyll and phycocyanin contents remained almost unchanged after infection. Host potential to operate cyclic photophosphorylation in the presence of phenazin methosulfate and non-cyclic phosphorylation was retained during the infection cycle. O_2 evolution did not change in the intact cells or in the cell-free preparation (lysed spheroplasts). The total ATP level in infected cells was identical to the level in non-infected cells.

TABLE III

PHOTOSYNTHETIC CAPACITY IN *PLECTONEMA BORYANUM* INFECTED WITH CYANOPHAGE LPP1G

Plectonema cells were infected with cyanophage LPP1G at input multiplicity of 10 plaque-forming units/cell; photosynthetic parameters were determined 6 h after adsorption. Values were expressed by the following units: CO_2 photoassimilation in μmol CO_2/mg cell protein per h from data in Fig. 1B (intact cells); μmol CO_2/mg protein per h (cell extract); non-cyclic photophosphorylation in μmol ATP/μatom O(P/2e); cyclic photophosphorylation in μmol ATP/mg chlorophyll per h; O_2 evolution in μmol O_2/mg chlorophyll per h; pigments in μg pigment/mg cell protein; total ATP in μg ATP/mg cell protein.

Parameter	Non-infected	Infected
CO_2 photoassimilation		
Intact cells	27.0	2.5
Cell extract (ribulose-1,5-diphosphate carboxylase)	8.4	8.4
Photophosphorylation in lysed spheroplasts		
Non-cyclic (P/2e)	1.00	0.9
Cyclic (phenazine methosulfate)	1000	1000
O_2 evolution		
Intact cells	828	810
Lysed spheroplasts	82	66
Pigments in cell extract		
Chlorophyll	12	11
Phycocyanin	172	140
Total ATP in cell extract	0.6	0.6

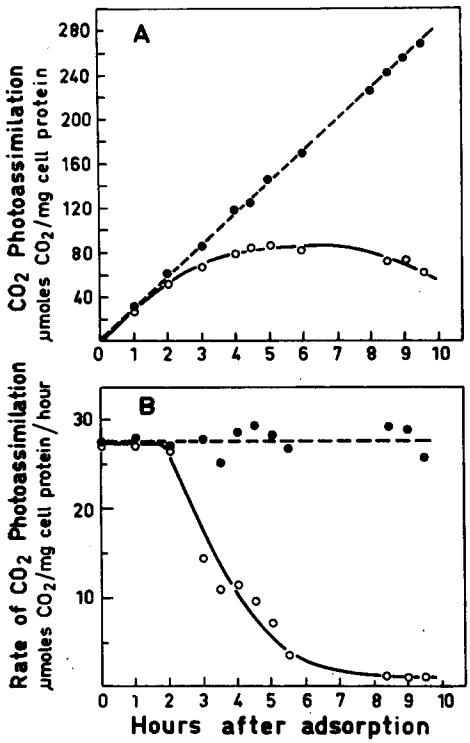

Fig. 1. CO_2 photoassimilation in *P. boryanum* infected with cyanophage LPP1G at input multiplicity of 10 plaque-forming units per cell. (A) Continuous incorporation of $NaH^{14}CO_3$. (B) Pulse labelling. ●—●, non-infected control; ○—○, infected cells.

Dependence of CO_2 photoassimilation inhibition on protein synthesis and on photosynthetic activity of Plectonema infected with cyanophage LPP1G

The relation of the inhibition of CO_2 photoassimilation to protein synthesis in infected cells was tested by following CO_2 fixation after addition of chloramphenicol. Chloramphenicol added to uninfected cells inhibited protein synthesis within 30 min and CO_2 photoassimilation after 3 h [1], therefore the chloramphenicol effect on the infected system could only be tested at 2 and 3 h after adsorption. Addition of chloramphenicol at these times to the infected systems did not affect the pattern of inhibition of the CO_2 photoassimilation. Assuming that chloramphenicol inhibits protein synthesis in the infected cells at the same rate as in uninfected cells, the early inhibition of CO_2 photoassimilation observed in the presence of chloramphenicol must be unrelated to protein synthesis.

To test whether photosynthetic activity is required for the phage-induced inhibition, infection was conducted in the dark using *Plectonema* cells having enhanced dark respiration, which allows a rate and efficiency of cyanophage replication in the dark close to that obtained in the light [1]. Inhibition of CO_2 photoassimilation occurred in these cells both when kept in the light or dark during the eclipse period of the infection cycle (Fig. 2). Uninfected cells showed constant CO_2 fixation rates when transferred to light, identical to that in continuous light. It seems

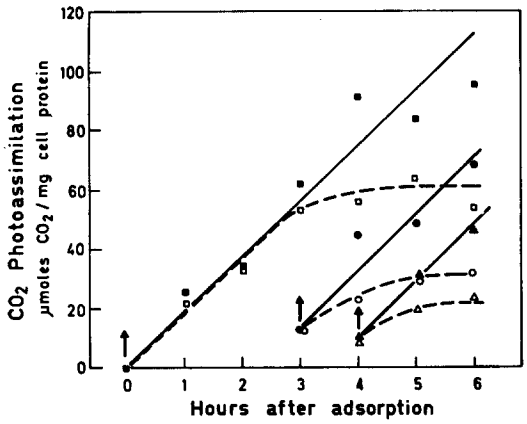

Fig. 2. CO_2 photoassimilation in *P. boryanum*, preincubated for 12 h in optimal photosynthetic conditions [1], infected with cyanophage LPP1G at input multiplicity of 10 plaque-forming units per cell. Cells were infected in the dark and illuminated immediately after adsorption (□—□) and 3 h (○—○) and 4 h (△—△) after adsorption. Black symbols, uninfected cells; white symbols, infected cells.

that the phage-induced inhibition of CO_2 fixation does not depend on photosynthetic activity during the eclipse period.

Plectonema cells can grow in the dark using ribose as substrate [18], and we found that ribose was incorporated as efficiently (1 μmol ribose/mg cell protein per h) in infected cells as in uninfected cells. CO_2 photoassimilation, determined in *Plectonema* cells preincubated in ribose (10 mM) for 12 h before cyanophage LPP1G infection in the presence of ribose, was inhibited as much as in infected cells not grown in ribose.

DISCUSSION

The only host photosynthetic parameter found affected by cyanophage LPP1G infection was the CO_2 photoassimilation in intact cells, which ceases during early stages of the infection cycle. The examination of in vivo CO_2 photoassimilation in infected *Plectonema* cells by following continuous incorporation (see Fig. 1A) and by the pulse technique (Fig. 1B) ruled out the possibility of incorporation and release of the labelled compounds. The finding that in vivo O_2 evolution functions in infected *Plectonema* cells at the same rate as in uninfected cells indicates unaltered photosynthetic activity in the infected cell although such cells are unable to fix CO_2. Such a condition demands regeneration of ADP and NADP not mediated by CO_2 carboxylation reactions. Apparently biosynthetic reactions induced by the cyanophage are responsible for the regenerated ADP and NADP utilized in the photosynthetic reactions.

From the evidence obtained here it seems that the CO_2 fixation cessation is not due to any major damage to the photosynthetic system. This cessation may be due to competition of the viral biosynthesis and the fixation reaction for ATP, NADPH and possibly also for other metabolic intermediates. Cessation of CO_2 photoassimilation may also be due to control of the activity of ribulose-1,5-diphosphate carboxyl-

ase either by the level of the substrates of the carboxylation reaction or by compounds in the internal milieu of the infected cell. It was shown that the activity of ribulose-1,5-diphosphate carboxylase in *Chlorogloae frischii* was inhibited by glycerophosphate or fructose [19]. It is also possible that the cessation of CO_2 photoassimilation is unrelated to the carboxylating enzyme since alternation in other essential enzymes or metabolites, in the infected cell may lead to the same result. In any event the inhibition of CO_2 photoassimilation on one hand and the unaltered functioning of other parameters of the photosynthetic system, on the other, imply that photosynthesis is shunted from use for host synthesis to viral synthesis.

Through the study of the dependency of cyanophage-infective processes on the host's bioenergetics it is possible to reveal the metabolic potencies of the host. In photoautotrophic conditions, the photosynthetic system is the main energy conversion system of *Plectonema* cells, and the respiratory system has little effect on host bioenergetics. Anaerobiosis does not alter the phage infection cycle under the photoautotrophic conditions [3]. The low phage burst size obtained in photoautotrophically grown cells transferred to the dark reflect the low level of endogenous respiration. Enhanced endogenous respiration induced by incubating the photoautotrophic cells in optimal CO_2 photoassimilation conditions is closely correlated to enhanced cyanophage yield [1]. *Plectonema* cells can grow heterotrophically on glucose; in such cells the cyanophage infection cycle is identical to that obtained under photoautotrophic conditions. Since anaerobiosis blocks phage replication in these heterotrophic conditions (see Table I), energy conversion by respiration is as efficient as photosynthesis for sustaining viral growth. Upon transfer of the photoautotrophic cells to these heterotrophic growth conditions, there is a lag of 17 h in the burst time of cyanophage LPP1G. This lag is fully correlated to the period required for induction of glucose uptake [2]. Only cyclic photophosphorylation is required for LPP1G cyanophage replication in autotrophic conditions [3]. Similar situation was found here for a serologically different [17] cyanophage LPP2SPI, which has a shorter burst time. The efficient Photosystem I-driven cyclic photophosphorylation demonstrated in infected *Plectonema* cells indicates that the cyanobacteria occupy a special position in the evolution of photosynthesis. This has been further amplified through the recent isolation of an *Oscillatoria limnetica* strain having Photosystem I-driven anoxygenic photosynthesis in which H_2S serves as the electron donor, which can shift to oxygenic photosynthesis [20]. In addition Rippka [21] found that it is possible to obtain Photosystem I-driven growth of many cyanobacteria.

Photosystem I-driven cyanophages LPP1G and LPP2SPI multiplication also demonstrates that this system produced progeny efficiently without an external carbon source. In this study we have shown that development of the cyanophage LPP1G in heterotrophically grown *Plectonema* cells depends on endogenous respiration, and does not depend on exogenous carbon source (glucose). Growth of the host cell is thus not required for cyanophage replication. In this respect the cyanobacterium - cyanophage system differs from systems involving bacteriophages of other facultative autotrophic bacteria in which phage replication is sustained only under very stringent conditions. The efficiency of bacteriophage SH 133 synthesis in autotrophically grown *Hydrogenomonas facilis* is much lower than in heterotrophically grown cells [22]. Although bacteriophage RC 1 replicates in the cells of the

photosynthetic bacterium *Rhodopseudomonas capsulata* growing photoautotrophically at almost the same efficiency as in cells growing in the dark, replication of this phage is completely inhibited in conditions where photosynthesis is depressed though the bacteria still grow [23].

Though the cyanophage N-1-*Nostoc* system [24] seems to resemble cyanophage LPP1G-*Plectonema* system, different patterns of viral-host interaction are shown by other cyanophages [25, 26]. Thus the cyanophage LPP1G interaction with *Plectonema* represents a special mode of host-virus interaction which can be visualized only in a photoautotrophic host such as *Plectonema*. In this host endogenous compounds can support viral growth based on respiration in the dark. In the light, inhibition of only one key reaction, the photoassimilation of CO_2, is sufficient to funnel the light-driven energy conversion system (both ATP and NADPH) from host biosynthesis to viral synthesis

The cyanophage LPP1G-*Plectonema* type of interaction seems to have a selective advantage as it is less dependent on environmental factors and less stringent with respect to the physiological state of the cell than other systems of cyanophages and bacteriophages. The wide distribution in nature of the LPP-type cyanophages [27], paralleling the world-wide distribution of the host, supports this assumption.

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