AS-1 cyanophage infection inhibits the photosynthetic electron flow of photosystem II in *Synechococcus* sp. PCC 6301, a cyanobacterium

Thomas A. Teklemariam, Sándor Demeter, Zsuzsa Deák, Gyula Surányi and György Borbély

Institute of Plant Physiology, Biological Research Center, H-6701 Szeged, Hungary

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In *Synechococcus* sp. cells AS-1 cyanophage infection gradually inhibits the photosystem II mediated photosynthetic electron flow whereas the activity of photosystem I is apparently unaffected by the cyanophage infection. Transient fluorescence induction and flash-induced delayed luminescence decay studies revealed that the inhibition may occur at the level of the secondary acceptor, Q_B of photosystem II. In addition, the breakdown of D_1-protein is inhibited, comparable to DCMU-induced protection of D_1-protein turnover, in AS-1-infected cells.

AS-1 Cyanophage; *Synechococcus* sp. PCC 6301; Cyanobacterium; Photosystem II

1. INTRODUCTION

Infection of a heterotrophic bacterial cell with bacteriophage is well known to result in a dramatically altered pattern of nucleic acids and protein synthesis in the host cell. It has been emphasized that the respiration of the heterotrophic hosts (the energy-yielding mechanism) is hardly affected by the bacteriophage infection up to the onset of lysis [1–4]. Therefore it has been assumed that the respiratory machinery of the heterotrophic host cell can supply energy and carbon skeleton in a suitable form and in a sufficient amount for the new synthetic processes initiated by bacteriophage attack.

The oxygen-evolving cyanobacteria are a major group of photosynthetic prokaryotes [5] and they are claimed to be phylogenetically, physiologically and functionally related to the chloroplast of photosynthetic eukaryotes. Most known cyanobacterial strains are obligate photoautotrophic and their metabolism is dependent on light as the only energy source for growth. Some members of the group are attacked by specific, DNA-containing cyanophages [6,7]. Therefore, an autotrophic cyanobacterium-cyanophage system seems promising to study the effect of virus infection on the photosynthetic and respiratory metabolism for a comparison with bacteriophage-infected heterotrophic cells. However, between unicellular and filamentous cyanobacterial hosts there is a marked difference in the dependence of cyanophage cycle on photosynthetic activity. In contrast to unicellular cyanobacterium-cyanophage systems, which show an absolute dependence for phage development on their host photosynthetic machinery (various *Synechococcus* strains and SM-1, AS-1 and AS-1M cyanophages) in filamentous organisms the cyanophage cycle can proceed independently of host photosynthesis (*Plectonema* sp. and *Nostoc* sp./*Anabaena* sp., LPP and N-1 phages [7]. The reason for this marked difference is not clear so far. However, as recently shown the altered redox state of thioredoxin *m* may give a possible explanation for this phenomenon in cyanophage-infected filamentous cyanobacteria [8].

It has been recently shown that in spinach chloroplasts infected by an RNA-containing virus the electron transport through photosystem II is inhibited [9]. The present work reports that infection of *Synechococcus* sp. PCC 6301, a unicellular cyanobacterium, by a DNA-containing cyanophage, AS-1 also results in the inhibition of photosynthetic electron flow of photosystem II at the level of the secondary quinone acceptor, Q_B.

2. MATERIALS AND METHODS

The AS-1 cyanophage and *Synechococcus* sp. PCC 6301 used and routine-culturing conditions have been described previously with a minor modification [6]. *Synechococcus* sp. cells ($3–5 \times 10^7$ cells ml^{-1}) were grown in Allen medium and infected by cyanophage AS-1 [6] at multiplicity of infection of 5–10 at 39°C as described [10]. Growth of the cultures was monitored either spectrophotometrically at 800 nm or by measurement of their chlorophyll-*a* content as described earlier [11,12]. The photosynthetic electron transport was estimated polarographically using a Clark type oxygen electrode at

Correspondence address: G. Borbély, Institute of Plant Physiology, Biological Research Center, PO Box 521, H-6701 Szeged, Hungary

Abbreviations: ASC, ascorbic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol-indophenol; MV, methylviologen; *p*BQ, *p*-benzoquinone; PS, photosystem; TMV, tobacco mosaic virus

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25°C [13]. For the assays 3 ml of cultures was removed in each half hour after infection and measured either (i) directly for the whole chain activity or (ii) assayed for PS II activity in the presence of 0.25 mM pBQ and (iii) for PS I activity in the presence of 8 μM DCMU, 100 μM DCPIP, 1 mM MV, 1 mM ascorbic acid and 2.5 mM NH_4Cl. Cells were briefly sonicated to ensure complete penetration of the reagents.

The procedures for in vivo fluorescence induction and for the delayed luminescence were essentially as described by Mohanty et al. [14,15]. For transient fluorescence induction and delayed luminescence measurements 16 ml and 50 ml of cell suspension were collected after cyanophage infection, respectively. Infected cells were collected by 1 min centrifugation in an SS-34 rotor of a Sorvall RC5C Du Pont centrifuge at 15000 rpm and 25°C. The cell pellet was resuspended in 3 ml of fresh medium kept at room temperature. If DCMU was present it was added at 15 μM final concentration. Fluorescence induction and delayed luminescence were excited by continuous light and by single flashes, respectively. All samples were dark-adapted for 5 min prior excitation [14,15].

For D_1-protein analysis, labeling and turnover were performed similar to Brusslan and Haselkorn [16] with the following changes: instead of radioactive methionine, 0.5 MBq ml^{-1} of ^{14}C-uniformly labeled protein hydrolysate was used for radiolabeling and the labeled proteins were autoradiographed as in [11].

3. RESULTS

The AS-1 cyanophage cycle was followed by measuring the absorbance of the infected cell suspension at 800 nm [10–12]. The optical density of the cell suspension is almost constant in the first 4 h and later it drops gradually due to the lysis of infected cells (Fig. 1). The effect of AS-1 on photosynthetic electron transport was examined by measuring the oxygen evolution in cyanophage-infected *Synechococcus* sp. cells. The whole-chain activity (H_2O-$NADP^+$) decreased to an undetectable low level during 4.5 h (Fig. 2A). In order to determine the inhibitory site of phage infection various segments of the electron transport chain were studied by the addition of electron acceptors and donors. The activity of photosystem II measured in the presence of parabenzoquinone, which accepts electrons at the secondary acceptor, Q_B continuously decreased throughout the cyanophage cycle and reached a 40% level at 4 h after infection. This inhibition of electron flow in AS-1-infected cells is essentially the same as observed in the absence of artificial acceptors (Fig. 2A and B). Contrary, no changes in the activity of photosystem I (DCPIP/ASC-MV) was obtained in the infected cells (Fig. 2C). Therefore, we concluded that the inhibition of photosynthetic electron flow of infected cells occurs between the water-splitting system and the secondary quinone acceptor, Q_B.

To analyse more closely the effect of AS-1 cyanophage infection on the photosystem II electron

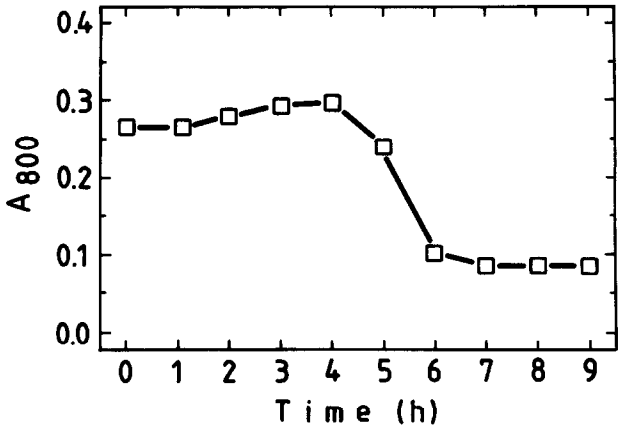

Fig. 1. Effect of AS-1 cyanophage infection on the growth of *Synechococcus* sp. PCC 6301. *Synechococcus* sp. cells were infected with AS-1 at a multiplicity of 10 and the growth was followed by reading A_{800} throughout the phage cycle.

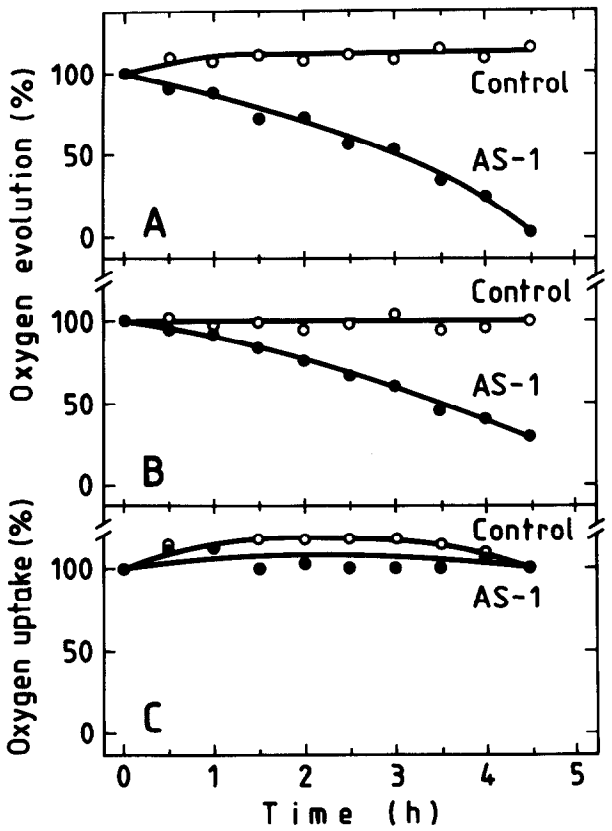

Fig. 2. Effect of AS-1 cyanophage infection on the oxygen evolution of *Synechococcus* sp. PCC 6301. (A) Changes of whole chain activity of photosynthetic electron flow in *Synechococcus* sp. cells after AS-1 infection. An uninfected culture was divided into two parts (zero time) and further cultivated without infection (control) and after AS-1 infection (AS-1), respectively. Whole-chain activity is expressed as % of control at zero time. The values are mean of 4 different measurements and corrected for chlorophyll-*a* content. At zero time 100% O_2 evolution: 190 μmol $O_2 \cdot$mg chl-a^{-1}. (B) PS II activity (H_2O-*p*BQ) of cyanophage infected cells. Experiments were performed as described for (A) but in the presence of 250 μm *p*BQ. At zero time 100% of oxygen evolution: 320 μmol $O_2 \cdot$mg chl-$a^{-1} \cdot h^{-1}$. (C) Photosystem I activity of AS-1 infected *Synechococcus* sp. cells. The assay was performed in the presence of 8 μmol DCMU, 100 μmol DCPIP, 1 mM MV, 2.5 mM NH_4Cl and 1 mM ascorbate. The values are mean of two separate experiments and corrected for chlorophyll-*a* content. The changes in oxygen uptake of the cells were obtained as a percentage of control. At zero time the oxygen uptake was 1207 μmol $O_2 \cdot$mg $chl^{-1} \cdot h^{-1}$.

transport we measured the chlorophyll fluorescence induction kinetics of those infected cells. This technique is a useful indicator of the impaired electron transport of photosystem II [17]. The fluorescence yield is determined by the redox state of the primary quinone acceptor Q_A [14,15]. Fig. 3 shows the transient fluorescence yield in healthy and infected cells either in the absence or presence of DCMU, respectively. At zero time the fluorescence level is the same in control and AS-1-infected cells. DCMU, which interrupts the electron flow at Q_B, enhanced the fluorescence equally in both cultures (Fig. 3A). In the early phase of cyanophage cycle, AS-1 phage infection itself gradually enhanced the fluorescence yield and at 2.5 h after the infection the fluorescence intensity reached the same level which is otherwise induced by DCMU (Fig. 3C). The enhanced yield of fluorescence due to phage infection suggests an inhibition of electron transport in photosystem II. In the later phases of cyanophage cycle a decrease in the fluorescence yield was observed even in the presence of DCMU (Fig. 3C and D). This may be the consequence of a general degradation of host cell constituents induced by the cyanophage genome.

It is noteworthy that the rise of fluorescence induction is the same in the phage-infected and in DCMU-inhibited control cyanobacterial cells (Fig. 2C and D).

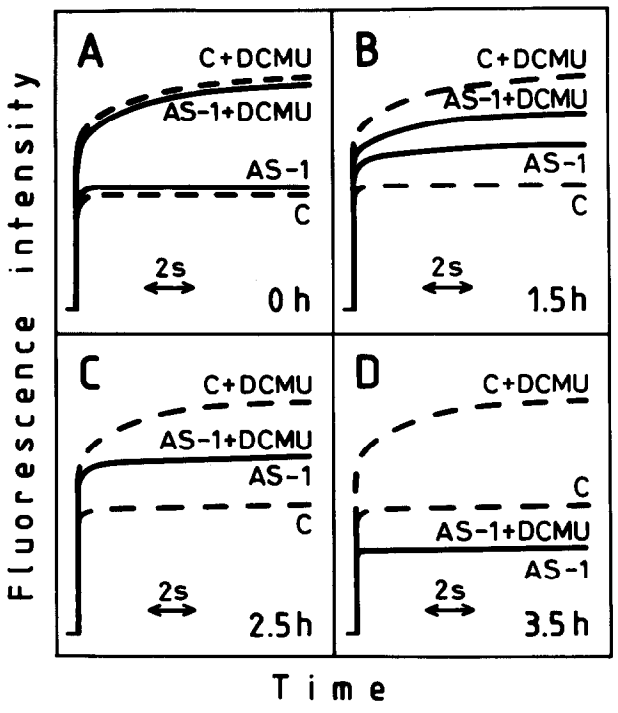

Fig. 3. Fluorescence yield transients of control (C) and AS-1-infected (AS-1) *Synechococcus* sp. cells at zero time and 1.5, 2.5, 3.5 h after cyanophage infection in the absence and presence of 20 μmol DCMU. The measurement was performed by continuous light excitation and fluorescence yield is given in arbitrary units. At 2.5 h the maximal fluorescence level is the same for the DCMU-treated control and AS-1-infected cells.

The resembling levels of fluorescence yield and the similar fluorescence rises in phage-infected and DCMU-inhibited cyanobacterial cells indicate a similar inhibitory site of DCMU and phage infection in the electron transport chain.

The action site of phage infection was further characterized by delayed luminescence measurements. Delayed luminescence originates from charge recombinations between oxidized donors and reduced acceptors of photosystem II. In the decay of delayed luminescence intensity two exponential components can be distinguished in the second time region [13]. The slow one with a half decay time of about 10 s belongs to charge recombination between the reduced secondary quinone, Q_B^- and the S_2 state of the water-splitting system. The fast-decaying component with a half time of about 2 s is associated with the $S_2Q_A^-$ charge recombination. If the electron transport is blocked between Q_A and Q_B the slow component is abolished and only the fast one appears in the delayed luminescence decay.

Phage infection of the *Synechococcus* sp. cells resulted in a considerable change in the delayed

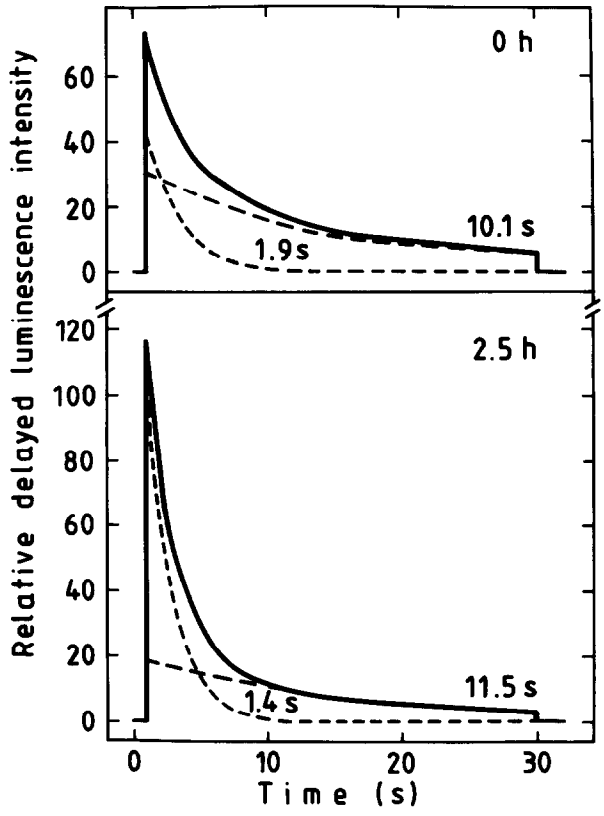

Fig. 4. Delayed luminescence decay of control and AS-1-infected *Synechococcus* sp. cells at zero time and 2.5 h after cyanophage infection. Measurements were performed by single flash excitation after 5 min dark adaptation of the samples. Solid line represents the experimentally measured decay course. The exponentially decaying delayed luminescence components obtained by computer-assisted curve resolution are shown by dashed lines. The numbers on the curves indicate the half-decay time of the components.

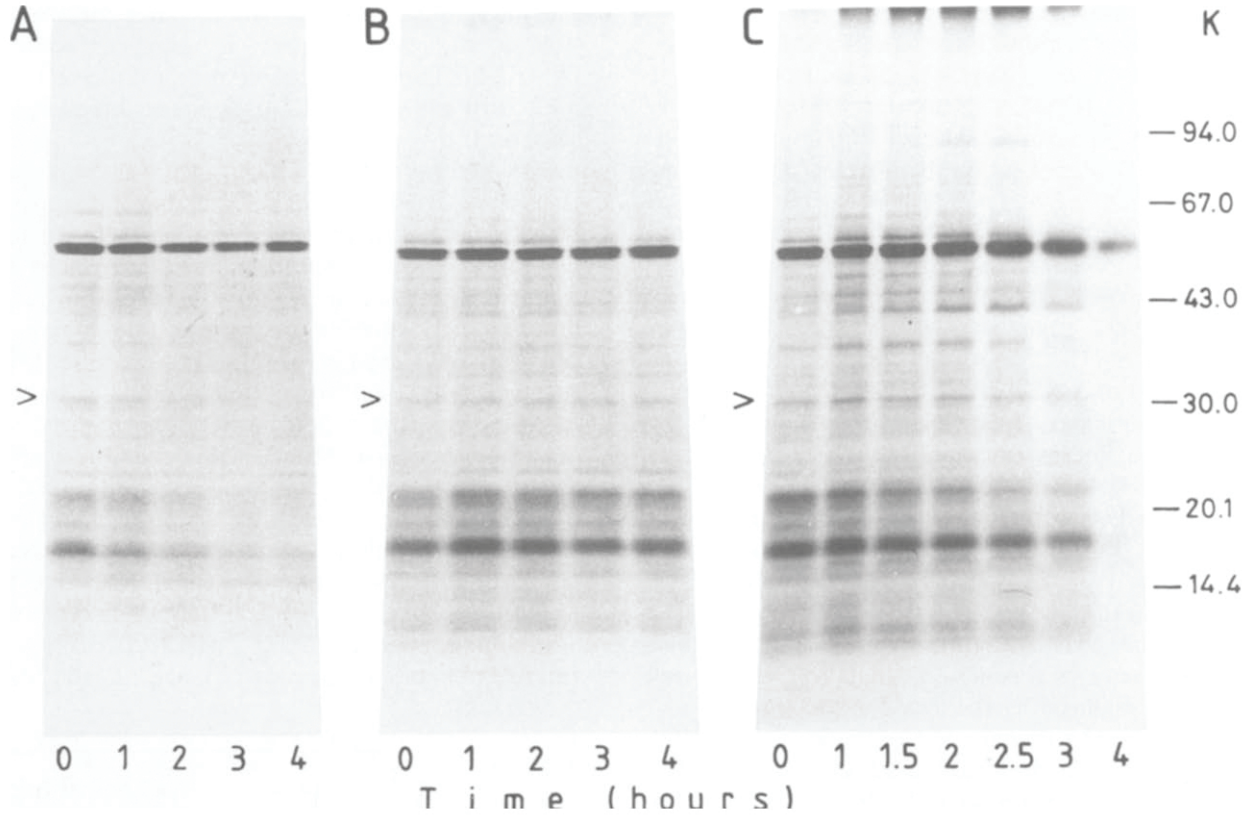

Fig. 5. Turnover of ^{14}C-labeled proteins in control (A), 10 μM DCMU-treated (B), and AS-1-infected *Synechococcus* sp. cells (C). Cells were labeled for 3 h as described in [16] and chased as shown. The D_1-protein is indicated by the arrow and the molecular mass of marker polypeptides is given in kilodaltons.

luminescence decay course. The amplitude of the slow component decreased approximately to half during 2.5 h of infection concomitantly with a two-fold increase in the intensity of the fast component (Fig. 4). This observation indicates a phage-induced inhibition of electron transport between Q_A and Q_B.

In an early attempt to elucidate the possible mechanism of AS-1-induced inhibition of photosystem II, at least at the level of photosystem II reaction center proteins we performed a radioactive chase experiment as described by Brusslan and Haselkorn [16]. As shown in Fig. 5, in control cells the D_1-protein turned over rapidly. DCMU (10 μM) inhibited the turnover of D_1-protein in a similar way as described in [16] and [18]. Surprisingly, AS-1 infection induced the inhibition of degradation of D_1-protein for 2.5 h and resulted in its higher stability (Fig. 5).

4. DISCUSSION

The results reported here demonstrate that AS-1 cyanophage infection results in a gradual inhibition of photosynthetic electron transport of *Synechococcus* sp. PCC 6301. While the electron transport of photosystem II is considerably inhibited at the level of the secondary quinone acceptor, Q_B, the activity of photosystem I is apparently not altered during phage infection. This latter observation is in accord with previous findings published for AS-1 [19], AS-1M [20] and SM-1 [21] cyanophage systems. The effect of phage infection on the photosynthetic electron flow is similar to the effects of other stresses like heat shock, heavy metal ion treatments and photoinhibition which restrain the photosynthetic activity due to specific alterations to photosystem II [14,15,22–25]. In addition it is a long tradition to treat bacteriophage infections as agents which interfere with cellular stress responses [26–28]. The present study extends to a DNA containing cyanophage-phototrophic cyanobacterium system, the inhibition of photosystem II by virus infection, a phenomenon which has been recently discovered in RNA-virus-infected plants [9,29]. However, there is an alteration from the TMV virus infected spinach chloroplasts in which the virus-induced rise of fluorescence is much slower than the DCMU-induced one [9,29].

The significance or the 'advantage' of the inhibition of photosystem II is not fully understood in AS-1 infected cells. It is generally accepted that the phage yield in infected unicellular cyanobacteria is strongly dependent on light [7,8,19]. Indeed, only 2% of normal AS-1 cyanophage yield is observed in dark, and under light condition in the presence of DCMU only 30% of phage yield produced in uninhibited control was obtained

[19]. The present finding, the gradual inhibition of photosynthetic electron flow of photosystem II, may be the consequence of the rearrangement of host cell metabolism by the cyanophage genome to produce phage particles. The activity of PS II seems to be sacrificed in phage-infected cells. We have shown that AS-1 cyanophage infection activates the oxidative pentose phosphate pathway (which is otherwise the main source of energy and reducing power in dark at the expense of stored glycogen [30]). The oxidative pentose phosphate pathway may provide enough energy and reducing power with the help of cyclic phosphorylation in preference to the whole chain photosynthetic electron transport. Consequently, data reported here would be of interest not only from the viewpoint of virology but it may provide a 'physiological' tool to study the photosynthetic electron flow of photosystem II.

This report demonstrates that the primary site of attack is at the level of the secondary quinone, Q_B. The binding site of Q_B is located on the D_1-protein of the photosystem II reaction center, possessing a very fast turnover rate. Any change in the synthesis or self assembly of this protein complex may adversely affect the electron transport of photosystem II [31,32]. Indeed, in AS-1-infected cells a higher stability of D_1-protein is observed, which is apparently similar to the DCMU-induced inhibition of D_1-protein degradation in cyanobacteria [16,18]. However, it remains to be clarified whether cyanophage-induced alterations at the Q_B site of D_1-polypeptide are responsible for the inhibition of electron transport or the observed lack of electron flow of photosystem II results in the altered stability of D_1-protein [16]. In order to elucidate the nature of phage-induced regulation of photosystem II at transcriptional and/or translational level we are currently studying the changes in infected cells.

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