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Effect of Light on the Attachment of Cyanophage AS-1 to *Anacystis nidulans*

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The effect of illumination on the extent and kinetics of the adsorption of cyanophage AS-1 to the blue-green alga (cyanobacterium) *Anacystis nidulans* was studied by using ^{32}P-labeled phage. The initial rate of adsorption was not significantly affected by light. However, at Na^+ levels used ordinarily to culture the alga ($[Na^+] = 11.7$ mM), the total amount of phage adsorbed was doubled in the illuminated cultures, as compared with the dark-grown ones, over a wide range of multiplicities of infection (0.05 to 20). Upon a 10-fold increase in Na^+ concentration in the medium ($[Na^+] = 0.11$ M), the dark adsorption of the phage increased to the level of light adsorption found in low Na^+ medium. The effects on phage adsorption of high Na^+ concentration and light were not additive.

The effect of light on a wide variety of processes involved in the reproductive cycle of cyanophages has been investigated in detail (1, 2, 4, 6, 7, 9–13). To our knowledge, the effect of light on cyanophage adsorption, however, has not been studied. In most published papers no mention is made of the conditions during the adsorption experiments. A few authors have explicitly stressed that the experiments had been carried out in the dark (11, 12) probably with the aim of securing more synchronous events upon illumination, after the fairly protracted adsorption process.

The present communication describes a phenomenon associated with, and possibly characteristic of the blue-green alga (cyanobacterium)/cyanophage system: the stimulatory effect of light on the adsorption of cyanophage AS-1 to *Anacystis nidulans* cells.

Organism, growth conditions, and purification of labeled AS-1 phage. A wild-type strain of *Anacystis nidulans* was grown as described previously (13) in a liquid medium containing the microelements of medium C and the major salts of medium D of Kratz and Myers (5). The $[Na^+]$ in this medium was 11.7 mM. Algal cells from the logarithmic phase were collected by centrifugation and resuspended in phosphate-free medium supplemented with 0.5 g of tris(hydroxymethyl)aminomethane per liter. After 4 h the cells were centrifuged and suspended in phosphate-free culture medium containing 0.5 g of tris(hydroxymethyl)-aminomethane, 10 mg of KH_2PO_4 per liter, and 50 μCi of $^{32}P_i$ per ml. After labeling for 8 h, the cultures were infected with AS-1 phage (8) at a multiplicity of infection of 0.5. The infected cultures were grown until lysis occurred. The method of Yamamoto et al. (14) based on the precipitation of the virus particles by polyethylene glycol was used for the purification of the labeled phage. The concentrated virus was dialyzed against cold culture medium to remove the free $^{32}PO_4^{3-}$ ions. The specific activity of the phage preparation obtained was 10^{-4} cpm/plaque-forming unit.

Adsorption experiments. Algae from the logarithmic phase were used (10^8 cells per ml). The labeled phages were added to 10-ml cultures at different multiplicities. The cultures were aerated by a stream of sterile air containing 5% CO_2. The dark cultures were wrapped in aluminum foil. The light cultures were illuminated with cool-white fluorescent light (3.6×10^4 mW/m^2). To avoid temperature differences, both the illuminated and dark cultures were thermostatically maintained at 37°C. Aliquots were withdrawn from the cultures at intervals and centrifuged, and the radioactivity of the nonadsorbed phages in the supernatant was determined by scintillation counting. To check the reliability of the isotope method, in some experiments the phage adsorption was tested by using the standard plaque assay (8) as well.

As shown in Fig. 1, the initial phase of phage adsorption was rapid. In the light 70% of the phage were adsorbed to the cells within 15 min. The further adsorption was slow, and at 80% adsorption a plateau was reached.

The adsorption of phages was similarly rapid in the dark for the first 6 to 8 min. In this case, too, the plateau of adsorption was reached after 20 min, but only about 40% of the phage were adsorbed to the cells in the dark. In a number of

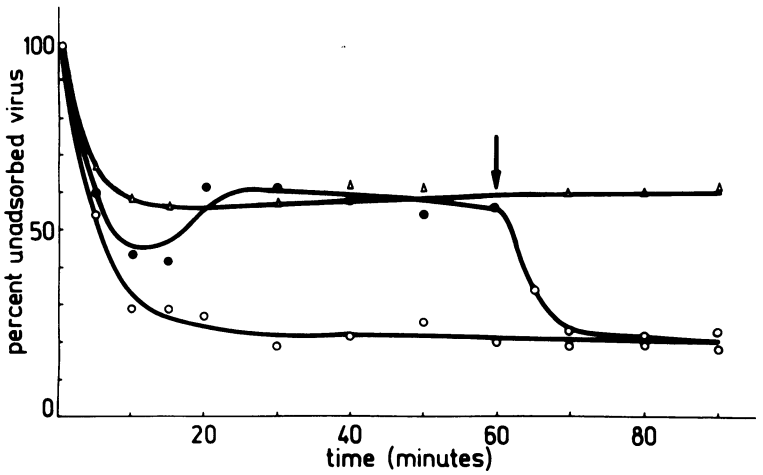

FIG. 1. *Effect of light on the adsorption of cyanophage AS-1 to Anacystis nidulans. The cells (1.0 × 10⁸/ml) were incubated with ^{32}P-labeled phage at a multiplicity of 3. Symbols: ○, cultures grown in the light and illuminated (3.6 × 10⁴ mW/m²) during phage adsorption; ●, cultures grown in the light but kept in the dark during phage adsorption; △, cultures kept in the dark for 12 h before infection and during adsorption. The arrow indicates the time of illumination of a dark culture.*

experiments, we observed an "overshoot" of phage adsorption in the dark (after 8 to 15 min of the adsorption process), i.e., the steady-state value of adsorption was reached only after a transitory maximum of phage attachment (Fig. 1).

If dark cultures were illuminated after the maximum adsorption level characteristic for the dark cultures had been reached, the cells started to adsorb more phage rapidly upon illumination, and after about 10 min phage adsorption was equal to that in the continuously illuminated cultures (Fig. 1).

The adsorption of phages to cells put into the dark at the time of the infection was compared with that of cells which had been incubated in the dark for 12 h before infection. The two samples behaved identically (Fig. 1).

The effect of light on phage adsorption was confirmed by plaque assay as well (Table 1).

The effect of light on the extent of phage adsorption was essentially the same at a wide range of multiplicities of infection (Table 2). This suggests that the phage population, in addition to a component the adsorption of which was increased in the light, contained another component the adsorption of which was unaffected by the light conditions.

The adsorption experiments described above were carried out in the medium used for culturing the alga (11.7 mM with respect to [Na^+]). Because higher NaCl concentrations favor the adsorption of AS-1 phage (8), the effect of light on phage adsorption was tested in the same medium supplemented with 0.1 M NaCl. The addition of 0.1 M NaCl increased the extent of adsorption in the dark approximately to the level of light adsorption in low Na^+ medium (Table 3). In the presence of 0.11 M [Na^+], the illumination had no effect on adsorption: the effect of high Na^+ levels and that of the light were not additive.

Earlier studies on the adsorption of cyanophages to their hosts have led to two major conclusions: (i) the adsorption process is qualitatively similar to that of the bacteriophages to

TABLE 1. *Phage adsorption in the light and the dark as determined by two different methods*[a]

Method	% Adsorbed phage		Light/dark ratio
	Light	Dark	
Plaque assay	94.1	61.0	1.5
Radioactivity assay	76.0	48.1	1.6

[a] Phage adsorption was determined in the same sample by plaque and radioactivity assays, respectively.

TABLE 2. *Phage adsorption at different multiplicities of infection*

MOI[a]	% Adsorbed phage[b]		
	Light	Dark	Light/dark ratio
20	63.8	36.0	1.77
5	70.0	33.9	2.06
0.05	64.2	32.7	1.96

[a] MOI, Multiplicity of infection.
[b] Determined by radioactivity assay.

TABLE 3. *Effect of Na^+ concentration on phage adsorption in the dark and in the light*

Na^+ ion concn in medium (M)	% Phages adsorbed[a]	
	Dark	Light
0.012	48.0	76.3
0.11	78.2	79.0

[a] Determined by radioactivity assay.

bacteria (1, 4, 8) and (ii) the adsorption process is slow (2, 4, 7, 11). It has also been shown that there is a considerable variation in the rate of adsorption (10).

The results presented in this paper show that with the cyanophage AS-1/*Anacystis nidulans* system the light conditions considerably affect the phage adsorption.

There are two observations which suggest that the effect is not a "nutritional" one. (i) The light effect was relatively fast, i.e., differences in the extent of adsorption in the illuminated and darkened cultures, respectively, were evident within 10 to 15 min. (ii) Cells cultured in light, or kept in darkness for 12 h before the experiments, behaved in the same way.

The effect of ions increasing phage adsorption is usually explained by charge neutralization permitting the phage to attach more firmly to the receptors. Light might exert a similar effect on the surface charges via photochemical reactions and/or light-induced changes in the immediate ionic milieu of the algae. In our experiments the effects of high Na^+ concentrations and light were not additive. This observation is compatible with, but does not necessarily support, the idea that the high Na^+ level and illumination affect the phage adsorption (ultimately) via a similar mechanism.

The Na^+ concentrations used in the experiments are well within the physiologically meaningful range, because Na^+ concentrations as low as 1 mg of NaCl per liter saturate the Na^+ requirement for growth of *Anacystis nidulans* and Na^+ levels as high as 2 g of NaCl per liter inhibit its growth rate only by 3% (3).

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