Ultraviolet Light Inactivation and Photoreactivation of AS-1 Cyanophage in Anacystis nidulans

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Black light effected photorecovery of AS-1 cyanophage and wild-type cells. However, only partial photoreactivation of AS-1 was observed in a partially photoreactivable mutant of Anacystis nidulans.

Photorecovery of ultraviolet (UV) radiation damage has been demonstrated in cyanobacteria (1, 8, 9) and cyanophage LPP-1 (9). Purified photoreactivation (PR) enzyme of Anacystis nidulans was reported to split pyrimidine dimers maximally at 436 nm (6). The UV absorption spectrum of purified enzyme showed a major protein peak at 275 nm and a small peak at 418 nm, but not at 436 nm (6). Upon further purification of the enzyme, a chromophore associated with the enzyme was revealed by its excitation (peak wavelength, 420 nm) and fluorescence (peak wavelength, 470 nm) spectra (3). These in vitro studies suggest that the PR enzymes play an important role in the photorecovery of UV-irradiated cells.

Recently, mutants of the cyanobacterium A. nidulans have been reported to demonstrate a lower level of photorecovery from UV radiation, as compared with the wild type (WT) (1). Analysis of partially photoreactivable mutants could yield information of photorepair systems in cyanobacteria. The cyanophage AS-1, isolated by Safferman et al. (5), permits investigation on the effects of UV radiation on the phage deoxyribonucleic acid (DNA) within its unicellular cyanobacterial host, A. nidulans. One could then test whether the partial PR characteristic of these mutants would result in partial PR of the cyanophage DNA. In this communication, I report the results of my studies on PR of AS-1 in the WT and in a partially photoreactivable mutant of A. nidulans.

Early infection stages were analyzed according to the procedure of Safferman et al. (5), with some modification. A. nidulans 625 served as the host, and infection was performed at 32 C. Cells were grown on Dm medium (7) under white fluorescent bulbs (Sylvania, F40 CWX). After 1 h, 71% phage adsorption was detectable, although maximal adsorption occurs at 2 h. The adsorbed cell fraction increased from zero time, maintained a plateau from h 1 and 2 after infection, and then showed a subsequent decrease. This decrease is expected, since a fraction of the infected cells begins to lyse. The eclipse phase is approximately 1 h under these conditions.

To analyze UV inactivation of cyanophage within the host cell, survival curves were determined on cyanophage alone, as compared with cyanophage DNA within the host. Dark-survival curves (in the absence of PR wavelengths) of free cyanophage and cyanophage within host cells are remarkably similar (see Fig. 1). Apparently little or no protection of cyanophage DNA is afforded by the host cell. For the dosage sufficient to inactivate 99% of phage, WT host cell inactivation is about 5%. This difference remains to be explained. The results demonstrate, however, that the PR of cyanophage DNA could be determined without involving other photorecovery systems in cyanobacteria.

PR experiments were conducted, and the results are shown on Fig. 2. The black light (fluorescent bulb, Sylvania BLB 40) used here has a major peak at 350 nm and a minor peak at 436 nm. The partially photoreactivable mutant, uvs-67, was isolated as described elsewhere (1). Note that phage growth of the unirradiated phage-host complex does not occur under black-light illumination (solid squares). Gold fluorescent light does not promote PR (open squares in Fig. 2A). The increases in plaque-forming units indicated for WT (solid circles) and for the partially photoreactivable mutant (open circles) are, therefore, ascribed to PR of UV-damaged phage DNA. Efficient PR of AS-1 DNA in WT cells and host cell DNA is observed (solid circles, Fig. 2A and B). In contrast, uvs-67 displays partial PR of cyanophage DNA, as well as partial PR of its own cellular DNA (open circles, Fig. 2A and B). Although not shown, partial PR of AS-1 was also obtained when AS-1 was irradiated before adsorption of host cells for determination of PR properties.

For the UV dosage applied, the data suggest that the PR of cyanophage DNA involved pho-
to repair of pyrimidine dimers and that a common enzyme is responsible for the PR of UV-damaged DNA of the host cells and cyanophage. Biochemical analysis of pyrimidine photorepair in determining the partial PR characteristic of these mutants is quite difficult (unpublished data) because of the low-level incorporation of labeled pyrimidines (4, 2). Two or more different interpretations could account for the partial PR of uvs-67. (i) The mutant is a "leaky" mutant, i.e., the enzyme retains some activity. (ii) A mutation has altered the active site of the PR enzyme. (iii) There is more than one distinct class of photorepair enzymes, and uvs-67 has lost the activity in one of them. It is not possible to distinguish these possibilities with the data that are presently available.

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LITERATURE CITED


**Anacystis nidulans** and **Synechococcus cedrorum**. Virology 47:105-113.


