Prophage Induction by Ultraviolet Light in *Acinetobacter calcoaceticus*

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UV-induction of prophage P78 of *Acinetobacter calcoaceticus* increased with the UV-dose given to the lysogenic strain from the spontaneous induction frequency of about 0.8% to a maximal frequency of 10%. This 10- to 20-fold increase of induction frequency, as measured by the number of infective centres, was accompanied by a 1000-fold increase in the yield of free phage. This effect was probably due to an increase in burst size under the conditions of lysogenic induction. Unusually, the lysogen was more resistant to UV-irradiation than the corresponding non-lysogenic strain.

INTRODUCTION

The complex response of *Escherichia coli* to UV-irradiation and other agents that damage DNA or inhibit its replication includes cell mutagenesis and filamentation, prophage induction, and Weigle-reactivation and Weigle-mutagenesis of the infecting phage. The response is controlled by the *recA* and *lexA* genes and is dependent on *de novo* protein synthesis (Witkin, 1976). Similar responses to DNA damage in other bacterial species have been described (Walker, 1984), but none of them is understood in such detail. The presence of a UV-inducible response in *Acinetobacter calcoaceticus* was suggested by Berenstein (1982) after the demonstration of Weigle-reactivation of the lysogenic phage P78. The UV-lysogenic induction of prophage P78 described here is further evidence for the presence of the UV-inducible system in *A. calcoaceticus* strain Ac.78.

METHODS

Bacterial strains and growth conditions. *A. calcoaceticus* strain Ac.78 and its lysogenic derivative Ac.78(P78) (Herman & Juni, 1974) were supplied by Dr E. Juni, University of Michigan, USA. Cultures were routinely grown to exponential phase at 37 °C in BPB (Difco).

UV-irradiation. Bacterial cultures at $OD_{570} = 0.4$ (about 1×10^8 cells ml⁻¹) were washed, resuspended in 0.01 M-MgSO₄ and, while being stirred continuously, were irradiated on ice at a UV-flux of 6×10^{-2} J m⁻² s⁻¹. The thickness of irradiated suspension was less than 1 mm. The UV-source used was a Sylvania germicidal lamp G8T5 emitting at 254 nm. The UV-dose was measured with a germicidal-erythral radiometer IL 570 (International Light).

Induction of the lysogenic phage. Ac.78(P78) was grown and irradiated as described above. At different times samples were withdrawn to be (a) plated to estimate c.f.u., (b) plated with indicator bacteria to estimate infective centres and (c) diluted 100-fold into BPB at 37 °C, shaken for 2 h and centrifuged; the number of free phage in the supernatant was then estimated by plating appropriate dilutions on plates of BPB plus indicator bacteria. Maximal values of p.f.u. were reached after 2 h post-irradiation incubation and there was no further increase with longer incubation time.

RESULTS AND DISCUSSION

The spontaneous induction frequency of prophage P78 from *A. calcoaceticus* strain Ac.78(P78) varied from 0.5 to 1% (Fig. 1). This value is close to the spontaneous induction

Abbreviation: BPB, Bacto Penassay Broth.

Fig. 1. Prophage induction in *A. calcoaceticus* strain Ac.78(P78) by UV-irradiation. The number of free phage in the supernatant (○) was determined after incubation of the induced culture in broth for 2 h. Induction frequency (●) is the number of infective centres, estimated after irradiation, relative to the number of c.f.u. present before irradiation. Burst size (▲) was calculated separately for each experiment as the ratio of free phage ml⁻¹ to the number of infective centres ml⁻¹ after the same UV-dose. Points represent mean values and SD values from three independent experiments.

frequency of 1 to 2% in *Staphylococcus epidermidis* (Silva & Leitão, 1984), but is 10- to 100-fold higher than the corresponding value of 0.01 to 0.1% of *E. coli* K12(λ) (Eitner, 1977; Witkin & Wermundsen, 1977). UV-irradiation of Ac.78(P78) caused no more than a 20-fold increase in the percentage of induced lysogenic cells. Induction frequency in terms of infective centres reached its maximum value of 10% for UV-doses from 1.8 to 7.2 J m⁻². This low efficiency of UV-induction of prophage P78 is striking when compared to other bacterial species: for example, in *E. coli* B/r(λ) 75% of lysogens were induced (Witkin & Wermundsen, 1977), and in *S. epidermidis* the efficiency of induction was close to 100% (Silva & Leitão, 1984). The limited UV-inducibility of prophage P78 cannot be caused by inactivation of the prophage itself, as at UV-doses up to 10 J m⁻² at least 80% survival of the vegetative phase was observed (Berenstein, 1982). At UV-doses over 7.2 J m⁻², the decreasing viability of the lysogenic cells and reduction of their phage-producing capacity could be the reason for the decrease in induction frequency. While UV-irradiation of Ac.78(P78) resulted in a maximum 20-fold increase in the number of lysogens forming infective centres, the phage yield increased about 1000-fold resulting in a maximum increase of burst size of 50-fold. A similar result has been reported by Notani & Setlow (1980) for *Haemophilus influenzae* and its phage HP1c1. In *H. influenzae* as well as in

Fig. 2. Comparison of UV-sensitivity of *A. calcoaceticus* strains Ac.78 (○) and Ac.78(P78) (●). Each point represents the mean value and SD calculated from at least three determinations.

A. calcoaceticus (Fig. 1), the peak of infective centres was much broader than that of the progeny phage and the effect of UV on the number of viable infective centres was much smaller than on the number of progeny phage.

How can the increase in UV-dose cause the increase in phage burst size? One possibility is that a regulatory protein inhibits directly or indirectly the replication of vegetative phage and is inactivated during UV-irradiation. Such a protein could be involved in DNA replication in P78 in a way that is analogous to the role of *lexA* protein for λ development in *E. coli* (Sprizhitsky & Kopylov, 1983). A two to threefold lower phage yield per induced cell was found in *lexA* mutants of *E. coli* compared to the wild-type lysogen (Carvalho & Leitão, 1984). Thus, enhanced cleavage of the putative *lexA* repressor of *A. calcoaceticus* could explain the increased burst size in the induced lysogens.

The better UV-survival of the lysogenic strain compared to the survival of the non-lysogen (Fig. 2) can be explained if P78 possesses its own UV-repair-genes that are expressed in the bacterial host, resulting in repair of both the bacteriophage and the bacterial DNA. The shoulder on the UV-survival-curve of Ac.78(P78) could be due to the protective effect of additional functions specified by P78. Genes of similar function have been reported in phage T4 (Harm, 1968), and in phage C5 of *Pseudomonas aeruginosa* (Dirckze *et al.*, 1979). Comparison of the frequency of prophage P78 induction (Fig. 1) with UV-survival of the lysogenic strain (Fig. 2) suggests that some of these cells lose their colony-forming ability without becoming infective centres. Accordingly, the maximum induction dose (7.2 J m⁻²) causes 10% of the cells to become infective centres, but leaves only 20% to survive. This may be caused by the UV-sensitivity of Ac.78 itself (non-lysogens give only 1% survival at this UV-dose). An alternative explanation may be that abortive induction is brought about by UV-irradiation in a majority of Ac.78(P78) cells.

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