Effects of Antipain (a Protease Inhibitor) on Respiration, Viability, and Excision of Pyrimidine Dimers in UV-Irradiated Escherichia coli Cells

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The protease inhibitor antipain increases the effectiveness of UV irradiation on cessation of respiration and cell killing in Escherichia coli B/r cultures without affecting excision of pyrimidine dimers. The actions are similar to those caused by cyclic AMP in irradiated cultures.

The protease inhibitor antipain [(1-carboxy-2 phenylethyl)carbamoyl-L-arginyl-L-valyl-arginal] (10, 11) prevents the induction of λ prophage in Escherichia coli by UV radiation (2). The induction involves proteolytic cleavage of the λ repressor (3), and the suggestion was made (2) that antipain inhibits the protease which carries out this cleavage.

UV-induced mutagenesis is also inhibited by antipain, and this inhibition is accompanied by reduced viability of the irradiated cultures (2). These results support the hypothesis of a UV-inducible error-prone repair operon regulated by a repressor that, upon induction, is cleaved by a protease (12). Induction of λ and mutagenesis are but two of a number of responses to UV that are dependent upon the recA* and lexA* gene products and are thought to involve inductions of operons (see references 5 and 12 for reviews). One of these responses is cessation of respiration, which begins 60 min after UV exposure and is associated with cell death (4, 7); both the respiration and lethal effects have been shown to be regulated by cyclic AMP (cAMP) (6).

We have studied the effects of antipain on respiration, viability, and excision of pyrimidine dimers in UV-irradiated E. coli B/r cells and have compared these effects with those produced in irradiated cells by cAMP. The results do not support the idea that cessation of respiration involves cleavage of a repressor by a protease. The cells were grown to midlog phase in liquid culture at 37°C in M63 medium with 0.04% glucose as a carbon source. Following an irradiation fluence of 52 J/m² at 254 nm, the cultures were incubated at 37°C in the presence and absence of 5 mM antipain, and respiration and viability were measured (see reference 5 and figure legends for details).

Figure 1 shows that UV irradiation (52 J/m²) caused a transient cessation of respiration, which we have shown is due to the dead cells that have had their respiration shut off irreversibly (4, 7). A graded series of concentrations of antipain caused progressively greater inhibition of respiration. A similar effect on the respiration of irradiated cells was previously found with cAMP (9). Figure 2 shows the growth patterns for irradiated and unirradiated cells in the presence of antipain. In Fig. 2A, antipain at a concentration of 5 mM caused UV-irradiated cells to stop growing at the same time respiration was shut off by the same concentration (Fig. 1). In Fig. 2B we show that growth of unirradiated cells was inhibited only slightly by antipain until about 200 min.

Figure 3 shows the time-course viability curves for irradiated cultures in the presence and absence of 5 mM antipain. The exponential increase of the irradiated cells in the absence of antipain is caused by division of the surviving cells. The antipain-treated irradiated cells kept pace with the untreated ones for 30 min, and then followed a loss in viability for another 30 min. Finally, the surviving cells increased exponentially at a very slow rate over the next several hours. The insert of Fig. 3 shows how cAMP affected the viability time-course curve (9); the initial rise and fall of viability was followed by an exponential increase at the same rate as for untreated irradiated cultures.

In Fig. 4 are compared the effects of cAMP and antipain on the excision of pyrimidine dimers from UV-irradiated cells. Over a 2-h period there was no significant difference in rate or extent of excision between them or untreated cells. Poor correlations between excision of pyrimidine dimers and viability after various pre-and postirradiation treatments (other than photoreactivation and caffeine) have been reported.
by ourselves and others (see reference 5 for review).

Our viability results are in agreement with those of Meyn et al. (2), who showed that in-
creased killing of UV-irradiated cells by antipain accompanies decreased mutagenesis. Increased killing is to be expected if this protease inhibitor prevents the induction of an error-prone operon.

We have previously shown that the cells that die after UV have their respiration shut off irreversibly (4, 7), and in this paper we show that antipain causes more cells to shut off their respiration and to die. These results do not support the idea that a repressor is cleaved by a protease during UV induction of an operon involved in cessation of respiration. If that were the case, irreversible cessation of respiration would occur in fewer rather than more cells, and fewer cells would be killed. Antipain and cAMP have similar effects on respiration and viability of irradiated cells, although the division of surviving cells is decreased in the case of antipain. Perhaps the loss of viability caused by antipain is more closely associated with cessation of respiration than with error-prone repair. We suggest that antipain may be affecting regulation of respiration of irradiated cells, possibly through stimulation of cAMP production.

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FIG. 1. Effects of various concentrations of antipain on respiration of UV-irradiated (52 J/m²) E. coli B/r cells. Oxygen consumption of 2-ml samples was measured at 37°C with a Gilson differential respirometer fitted with photocells and drive mechanisms and interfaced with a PDP11 computer. The lines are tracings of data printed at a video computer.

FIG. 2. Effects of antipain (5 mM) on growth of irradiated and unirradiated cells. (A) Unirradiated (no UV), irradiated (52 J/m²) cells. (B) Unirradiated cells. For (B) the cultures were diluted several times during the 5-h period, and the successive displaced curves were aligned so as not to reflect the dilutions.
Fig. 3. Time-course viability curves for UV-irradiated E. coli cells in the presence and absence of antipain. After irradiation (52 J/m²), antipain (final concentration, 5 mM) was added, and the cells were incubated at 37°C. Cell dilutions were placed on glucose minimal medium solidified with 1.2% agar and incubated at 37°C for 48 h. Inset, from reference 8, shows the viability curves for irradiated cells (52 J/m²) incubated with cAMP.
Fig. 4. Excision of thymine dimers from UV-irradiated cells (52 J/m²) treated with antipain (5 mM) and cAMP (5 mM). Cells were labeled by incubating a culture of exponentially growing cells (4 × 10⁶/ml) for two generations with [³H]thymidine (17 Ci/mmol, 13 μCi/ml) in the presence of adenosine (final concentration, 0.28 mg/ml). Culture samples (0.2 ml) were assayed for the fraction of thymidine label remaining in dimers in DNA by the method of Carrier and Setlow (1).

Literature Cited