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Inhibition of Deoxyribonucleic Acid Repair in *Escherichia coli* by Caffeine and Acriflavine After Ultraviolet Irradiation

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The effects of caffeine and acriflavine on cell survival, single-strand deoxyribonucleic acid break formation, and postreplication repair in *Escherichia coli* wild-type WP2 and WP2 *uvrA* strains after ultraviolet irradiation was studied. Caffeine (0.5 mg/ml) added before and immediately after ultraviolet irradiation inhibited single-strand deoxyribonucleic acid breakage in wild-type WP2 cells. Single-strand breaks, once formed, were no longer subject to repair inhibition by caffeine. At 0.5 to 2 mg/ml, caffeine did not affect postreplication repair in *uvrA* strains. These data are consistent with the survival data of both irradiated WP2 and *uvrA* strains in the presence and absence of caffeine. In unirradiated WP2 and *uvrA* strains, however, a high caffeine concentration (>2 mg/ml) resulted in gradual reduction of colony-forming units. At a concentration insufficient to alter survival of unirradiated cells, acriflavine (2 μg/ml) inhibited both single-strand deoxyribonucleic acid breakage and postreplication repair after ultraviolet irradiation. These data suggest that although the modes of action for both caffeine and acriflavine may be similar in the inhibition of single-strand deoxyribonucleic acid break formation, they differ in their mechanisms of action on postreplication repair.

Pyrimidine dimers are the principal photoproducts formed in DNA of bacterial cells after UV irradiation (1, 17). Subsequent removal of the dimers by excision repair is crucial to the survival of the irradiated cells. In *uvrA* cells that lack excision repair capacity, these cells can circumvent UV damages by another repair pathway called postreplication repair (14, 15).

Excision repair of pyrimidine dimers involves principally four enzymatic steps: (i) UV-specific endonuclease recognizes the dimers and makes an incision break at the 5′ ends of the dimers; (ii) DNA polymerase I 5′ → 3′ exonuclease degrades the damaged portions; (iii) polymerase I resynthesizes the DNA through the opposite intact DNA templates; and (iv) ligase seals off the resynthesized DNA. Postreplication repair entails the transfer of parental DNA to fill in the daughter strand gaps formed opposite the unrepaired pyrimidine dimers (15). This repair mechanism, as it occurs in *uvrA* cells, does not result in removal of pyrimidine dimers, but rather the dimers are diluted out through subsequent DNA replication to produce dimer-free daughter strands (7).

Both caffeine and acriflavine were employed to delineate the relation between inhibition of repair of pyrimidine dimers produced by UV irradiation and cell lethality, since these two compounds were shown to cause enhanced UV killing and reduced levels of excision repair in bacterial cells (9, 18–20, 22). However, there were few data to demonstrate the step(s) underlying the action of caffeine and acriflavine by which excision repair is inhibited. It is also not known whether these two compounds would have different modes of action on the inhibition of repair of pyrimidine dimers caused by UV irradiation. Using nonlethal doses of caffeine and acriflavine, we examined their effects on the incision step of the excision repair pathway in a wild-type WP2 strain and on the postreplication repair pathways in a *uvrA* strain.

Caffeine, at a concentration of 2 mg/ml and lower, did not appreciably alter the survival of unirradiated wild-type WP2 and *uvrA* cells (Tables 1 and 2). Figure 1A and B show the effects of caffeine on the formation of single-strand DNA breaks when it was added before and after UV irradiation. When caffeine (0.5 mg/ml) was incubated with WP2 cells for 30 min before irradiation, there was virtually no single-strand DNA breakage. Single-strand breaks were also prevented, albeit partially, by adding caffeine immediately after UV irradiation. Repair of the incision breaks was not affected since further incubation for 50 min led to the restoration of molecular weights similar to those of the unir-

TABLE 1. *Survival of irradiated and unirradiated WP2 cells in the presence of caffeine and acriflavine*

Additive concn	Survival[a] after UV dose of:			
	0 J/m²	10 J/m²	20 J/m²	30 J/m²
Caffeine (mg/ml)				
0.00	1.00		0.70	0.60
0.50	1.00		0.60	0.45
2.00	0.98		0.57	0.43
3.00	0.65		—[b]	—
3.50	0.37		—	—
4.00	0.00		—	—
Acriflavine (μg/ml)				
0.00	1.00	0.97	0.69	0.58
2.00	0.98	0.45	0.070	0.02
5.00	0.38	—	—	—

[a] Colony-forming units per milliliter.
[b] —, Not determined.

radiated control. Thus, it can be concluded that caffeine inhibits the incision step leading to single-strand DNA break formation. DNA single-strand breaks, as detected in alkaline sucrose gradients, were formed very rapidly after UV irradiation. Only if caffeine were present at the time of irradiation and immediately after would the formation of these breaks be prevented. Once single-strand breaks are formed, they are no longer subject to repair inhibition by caffeine. Recently, a dimer-specific endonuclease was isolated by Braun and Grossman (2). Subsequent studies with dimer-specific endonuclease suggest that in the presence of caffeine (25 mM; 4.9 mg/ml), this enzyme is competitively inhibited (3). Thus, it could be envisaged that caffeine binds tightly to the irradiated DNA, thereby competing with dimer-specific endonuclease for the dimers.

UV killing was enhanced in wild-type WP2 but not in WP2 *uvrA* cells (Tables 1 and 2). This is consistent with the finding that caffeine (0.5 mg/ml) did not affect postreplication repair in *uvrA* cells (Fig. 1C). Postreplication repair was not inhibited at an even higher concentration (2 mg/ml) of caffeine. These results are in full agreement with those of McCulley and Johnson (11), who showed that 2 mg of caffeine per ml does not inhibit postreplication repair in *uvrA* cells. The failure of inhibition of postreplication repair by caffeine in normal human fibroblasts has also been described (10). This suggests that the underlying mechanism in postreplication repair in human cells may be similar to that in bacteria. However, it must be noted that the sensitivity of postreplication repair to caffeine in mammalian cells could be affected by whether the cells are from primary cultures or from established lines (6).

Acriflavine (2 μg/ml) caused very little lethality to unirradiated WP2 and *uvrA* cells. After UV irradiation, however, both WP2 and *uvrA* cells underwent enhanced killing. At 5 μg of acriflavine per ml, survival was reduced further. Acriflavine (2 μg/ml), when added before and immediately after UV irradiation, resulted in strong inhibition of single-strand DNA breakage, as was the case with caffeine (Fig. 1D and E). However, acriflavine did not seem to interfere with repair of incision breaks. In contrast to the effects of caffeine, acriflavine inhibited postreplication repair (Fig. 1F). Therefore, acriflavine differs from caffeine in that the former inhibits not only the formation of single-strand DNA breaks, but also postreplication repair. This finding explains the enhanced killing by UV in both wild-type WP2 and *uvrA* strains in the presence of acriflavine. Inhibition of single-strand DNA breakage by acriflavine could be similarly mediated through inhibition of dimer-specific endonuclease. If this were the case, the mechanism whereby postreplication repair is inhibited could be distinct from that inhibiting

TABLE 2. *Survival of irradiated and unirradiated WP2 uvrA cells in the presence of caffeine and acriflavine*[a]

Additive conc	Survival[b] after UV dose of:			
	0 J/m²	2.5 J/m²	5 J/m²	10 J/m²
Caffeine (mg/ml)				
0.00	1.00		0.17	0.008
0.50	1.00		0.17	0.009
2.00	0.95		0.16	0.008
3.00	0.71		—[c]	—
3.50	0.35		—	—
4.00	0.00		—	—
Acriflavine (μg/ml)				
0.00	1.00	0.85	0.20	0.009
2.00	0.97	0.38	0.08	0.0001
5.00	0.45	—	—	—

[a] Cultures were grown to a density of about 3×10^8 cells per ml in M-9 medium (4). The cells were then filtered onto membrane filters (Millipore Corp.) suspended in M-9 buffer to a final density of about 2×10^8/ml. Before UV irradiation, 0.1-ml amounts were pipetted onto minimal agar supplemented with 0.02% nutrient broth (Difco Laboratories) for determination of cell titer. The same procedures were repeated for scoring cell viability after exposure to different UV fluencies. Colonies were counted after 48 h of incubation at 37°C.
[b] Colony-forming units per milliliter.
[c] —, Not determined.

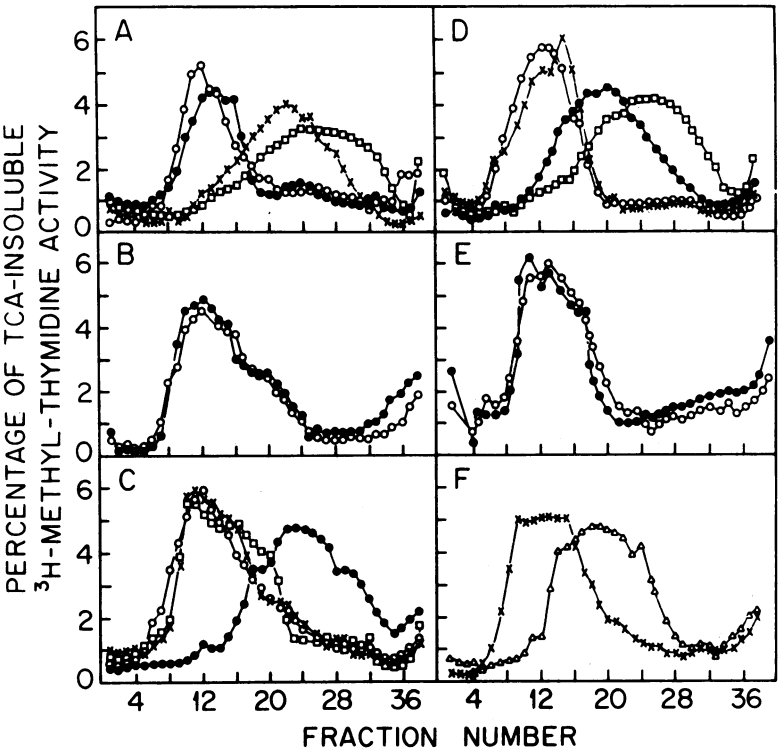

FIG. 1. *Effects of caffeine and acriflavine on the formation of single-strand DNA breaks and postreplication repair in E. coli strains. The procedures have been described previously (4, 5). Briefly, bacterial cells were labeled with [methyl-³H]thymidine (10 mCi/ml), filtered onto Millipore filters, and suspended in M-9 buffer before exposure to UV irradiation. Production of single-strand DNA breaks was measured by the distribution profiles of labeled DNA in alkaline sucrose gradients. Repair of DNA was confirmed by the conversion of initially low-molecular-weight DNA into large-molecular-weight DNA similar to that in unirradiated DNA. For measurement of postreplication repair, cells in M-9 buffer were irradiated and incubated for 10 min in fully supplemented M-9 medium with [methyl-³H]thymidine (100 mCi/ml) and 2-deoxyadenosine (150 μg/ml). The cells were then filtered onto Millipore filters and suspended in complete M-9 medium for further incubation at 37°C. Postreplication repair was verified by observing the change of pulse-labeled DNA into large-molecular-weight DNA similar to that of DNA labeled for 10 min in unirradiated cells. (A,B,D,E) Single-strand DNA breaks. (C,F) Postreplication repair. (A) Strain WP2: unirradiated control (○); 60 J/m² plus incubation for 10 min at 37°C (□); 60 J/m² plus incubation in caffeine (0.5 mg/ml) for 10 min (×) and for 60 min (●). (B) Strain WP2: unirradiated control (○); preincubation in caffeine (0.5 mg/ml) for 20 min at 37°C plus 60 J/m² (●). (C) uvrA strain: unirradiated control plus 10-min pulse-label (○); 10 J/m² plus 10-min pulse-label (●); 10 J/m² plus 10-min pulse-label, incubated for 60 min in caffeine at 0.5 mg/ml (×) and at 2 mg/ml (□). (D) Strain WP2: unirradiated control (○); 60 J/m² plus incubation for 10 min at 37°C (□); 60 J/m² plus incubation in acriflavine (2 μg/ml) for 10 min (●) and for 60 min (×). (E) Strain WP2: unirradiated control (○); preincubation in acriflavine (2 μg/ml) for 30 min at 37°C plus 60 J/m² (●). (F) uvrA strain: unirradiated control plus 10-min pulse-label (×); 10 J/m² plus 10-min pulse-label and incubation in acriflavine (2 μg/ml) for 60 min (△). Independent experiments demonstrated that, in (C) and (F), pulse-labeled DNA profiles of irradiated uvrA cells corresponded to those of unirradiated control after incubation for 60 min. TCA, Trichloroacetic acid.*

single-strand DNA breakage. Based on the data of several investigators (12, 14, 16, 21), Rothman and Clark (13) have distinguished three types of postreplication repair: (1) recombinational, (2) mutagenic, and (3) excisional. It is possible that acriflavine affects only recombinational and excisional postreplication repair without concomitantly inhibiting the mutagenic type. Indeed, UV mutagenesis in *uvrA* strains was enhanced by acriflavine (unpublished data; 20), although it also caused significant lethality (Table 2). On the contrary, caffeine may not inhibit the recombination type and its inhibitory effects are directed on the mutagenic and excisional types. This would explain why caffeine has a lethal effect on the wild-type WP2 strain (Table 1) but not on the *uvrA* strain (Table 2). Additionally, antimutagenic effects by caffeine in bacteria

have been reported by Clarke (4).

With caffeine concentrations of 3 and 3.5 mg/ml, colony-forming units of unirradiated WP2 cells were reduced by 35 and 63%, respectively, and of *uvrA* cells by 29 and 65%, respectively (Tables 1 and 2). Virtually no colony-forming units were seen at a concentration of 4 mg/ml. Grigg (8) found that using a caffeine concentration of 8 mM (1.6 mg/ml) resulted in 30 to 50% lethality in unirradiated *Escherichia coli* B and 15. It was at a caffeine concentration higher than 2 mg/ml that we saw gradual reduction in colony-forming units of unirradiated WP2 and *uvrA* cells. This comparison indicates that there are differences among bacterial strains in their sensitivities to caffeine. These observed differences could be due to differences in uptake of caffeine or the repair capacities of the bacterial strains in the presence of caffeine. However, Harm (9) dismissed these two possibilities and proposed that the *fil*$^+$ genotype, causing filament formation after UV irradiation, seems to be related to increased sensitivity to caffeine. He found that the action of caffeine and acriflavine on unirradiated DNA in cells is reversible because unirradiated cells of B or B_{s-1}, kept for 1 to 2 h in acriflavine and caffeine, were found to remain fully viable if plated afterwards with no caffeine and acriflavine. The reduction of colony-forming units seems to be a result of a varying degree of suppressed growth of the bacterial strains. At any event, caution should be taken in accounting for the effects of caffeine on DNA repair in irradiated cells, since at a caffeine concentration high enough to cause growth-suppressing effects in unirradiated cells, this observation might lead to erroneous conclusions.

In summary, it was found that at a concentration that did not alter the survival of the bacterial strains, caffeine inhibited single-strand DNA break formation, whereas acriflavine inhibited both single-strand break formation and postreplication repair. However, once single-strand breaks were formed, caffeine and acriflavine were no longer effective in the inhibition of DNA repair. Since the survival of unirradiated bacterial cells is sensitive to a high concentration of the two repair inhibitors, it is imperative that the concentration being used be taken into account in assessing the effects of caffeine and acriflavine on DNA repair in bacterial cells.

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