ACRIFLAVINE MODIFICATION OF NUCLEIC ACID FORMATION, MUTATION INDUCTION AND SURVIVAL IN ULTRAVIOLET LIGHT EXPOSED BACTERIA

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Witkin (1958, 1961) described an effect of acriflavine, caffeine and certain DNA combining basic dyes in increasing the magnitude of mutation frequency response to ultraviolet light in Escherichia coli. In the study described here, we compared the effect of these agents on RNA, DNA and protein synthesis, survival and mutation induction with UV-exposed bacteria. The results establish that the agents exert some action on UV-exposed cells which results in increased mutation frequency response and lethality, as well as blockage of RNA, DNA and protein synthesis. The effects of acriflavine on macromolecular synthesis, mutation induction and lethality is described in this report. A subsequent report will describe identical effects of caffeine and the DNA combining dyes on UV-exposed bacteria.

MATERIALS AND METHODS

E. coli strain WP2 (tryptophan requiring) is used. The strain was isolated by Dr. Evelyn Witkin from E. coli B/r after UV exposure and supplied to this laboratory some years ago. Culture growth procedures have been described (Doudney and Young, 1962; Doudney, 1963). Log phase cells are obtained by growth from a small inoculum at 37°C in minimal medium with L-tryptophan (20 μg/ml) in large Erlenmeyer flasks on a rotary shaker. Growth is to an optical density of 0.25 measured by a Bausch and Lomb "Spectronic 20" spectrophotometer in 1.8 cm optical tubes at a wave length of 660 mμ. The culture is resuspended in ice cold minimal medium free of tryptophan after washing on a Millipore

filter. Ten ml samples are exposed in cold 140 mm diameter petri dishes with magnetic stirring to UV radiation from a General Electric germicidal lamp. The dose given below 2,800 Å, as measured by a Hanovia model AV-971 UV meter at the position of the suspension, was about 200 ergs/mm^2. The UV-exposed bacterial suspension is diluted with 37°C minimal medium appropriately supplemented with tryptophan to give a final concentration of 20 μg/ml and then placed on a rapidly rotating shaker at 37°C. Ten ml samples are taken at appropriate intervals of time for RNA, DNA and protein determinations. The techniques used for RNA, DNA and protein determination have been described previously (Doudney and Young, 1962; Doudney, 1963).

Appropriate dilutions are made from samples of the culture which were taken with incubation after UV-exposure. These samples are plated on minimal agar supplemented with 2.5% nutrient broth. One-tenth ml of the final suspension is pipetted onto the agar surface and spread with a glass spreader. Induced mutation frequency is calculated as described by Doudney and Young (1962) and expressed as the number of prototrophic colony-forming organisms observed with 10^5 surviving auxotrophic colony-forming organisms appearing after three days incubation at 37°C.

RESULTS

At the concentration used, acriflavine has little effect on DNA synthesis in non-UV-exposed cultures (Figure 1). If the culture is exposed to UV before incubation with acriflavine, DNA synthesis is blocked for over 90 minutes. In UV-exposed cultures without acriflavine there is a 45 minute lag in DNA synthesis followed by complete recovery of synthetic capacity. Similarly, acriflavine has little effect on RNA synthesis in a non-UV-exposed culture (Figure 2). If exposed to UV, the culture has the capacity in acriflavine for a doubling of RNA; then synthesis ceases. Similar results have been observed with protein synthesis.

At the dose of UV used, incubation in the presence of acriflavine increases mutation frequency response from about 5 per 10^5 to 13 per 10^5 surviving organisms (Figure 3). This increase appears to be correlated with RNA synthesis in the culture. During the first 15 minutes of incubation, there is no change in

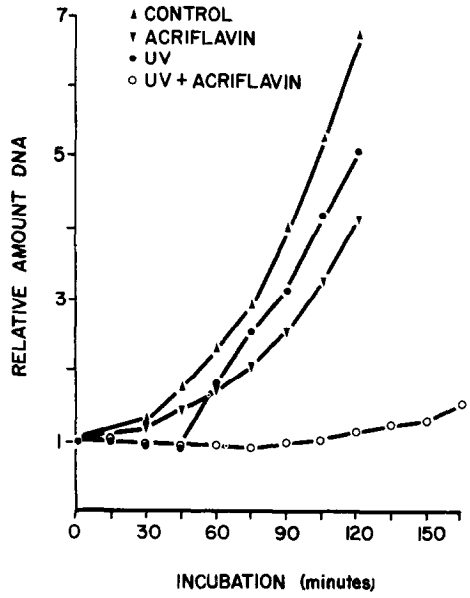

Figure 1.

Effect of acriflavine (1 μg/ml) on DNA synthesis in UV-exposed and control cultures of E. coli strain WP2. 1 = 32 μg of DNA per ml of culture.

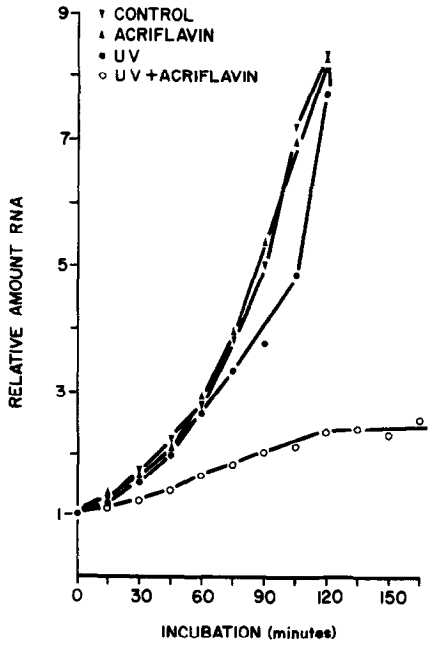

Figure 2.

Effect of acriflavine (1 μg/ml) on RNA synthesis in UV-exposed and control culture of E. coli strain WP2. 1 = 186 μg of RNA per ml of culture.

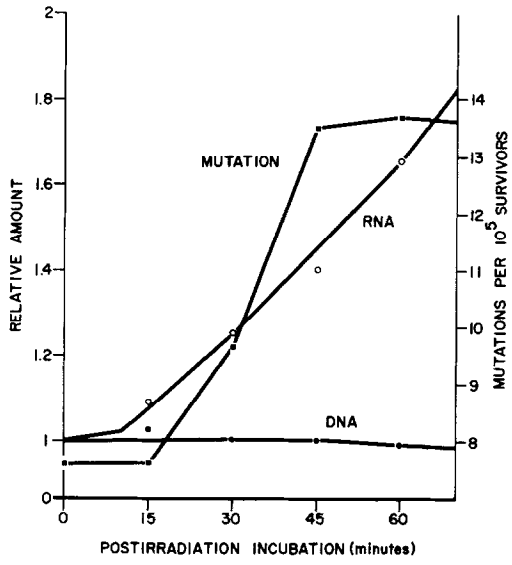

Figure 3.

Effect of acriflavine (1 μg/ml) on mutation frequency response with UV-exposed E. coli strain WP2 in comparison to synthesis of RNA and DNA in the UV-exposed culture. RNA: 1 = 188 μg/ml of culture; DNA: 1 = 32 μg/ml of culture. Acriflavine is not measurably mutagenic with non-UV-exposed cultures.

mutation frequency in acriflavine and little RNA formation. Upon initiation of RNA synthesis at about 15 minutes, a marked increase in mutation frequency occurs. No DNA is formed in the culture during the period of increasing mutation frequency response in acriflavine.

Incubation in acriflavine after UV exposure also decreases survival (Figure 4). This effect also appears to be correlated with RNA synthesis in the culture, as shown by subsequent studies not described here. (Subsequent studies have also shown that the concentration of acriflavine producing maximum effect is identical for inhibition of RNA, DNA and protein synthesis and increase in mutation frequency and lethality, suggesting an identical site of action for all effects. These studies will be described elsewhere.)

DISCUSSION

The results are consistent with a hypothesis in which acriflavine (known to interact with DNA [Lerman, 1963]) reacts with a UV-damaged site on the DNA

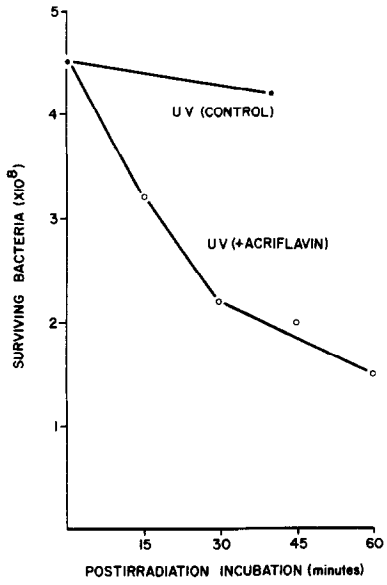

Figure 4.

Effect of acriflavine (1 μg/ml) on survival of UV-exposed E. coli strain WP2 with postirradiation incubation. Acriflavine has no measurable lethal effect with non-UV-exposed cultures.

(possibly the UV-induced thymine dimer [Setlow and Setlow, 1962; Ibid., 1963]) which is otherwise repairable, to prevent replication. This reaction would prevent formation on the DNA template of the component of the RNA synthetic system (presumably the messenger RNA) necessary for total RNA synthesis. The fact that RNA can double in the culture in the presence of acriflavine would suggest 1) that the cell has at all times enough gene action product for one round of RNA replication (assuming that the acriflavine reaction with damaged DNA is immediate) or 2) that it is the gene action event itself (messenger RNA formation) which "uncovers" the DNA to the inhibitory action of the acriflavine on the damaged site. This would allow one round of RNA formation before inactivation of the DNA template. The latter possibility is supported by the acriflavine effect on mutation induction.

In view of the correlation of both the observed increased lethal and mutagenic effects with RNA synthesis in the culture, it can be proposed that

the interaction of acriflavine with the UV-damaged site responsible is uncovered by RNA synthesis. Thus, when the damaged site on the DNA interacts with acriflavine after RNA synthesis, the mutagenic, lethal and metabolic effects are observed. The reaction of the dye would appear to be specifically with the DNA damaged site, explaining the fact that only UV damaged cells show these effects in the presence of the dye.

An interpretation along the same lines can be developed for mutation induced by UV (in the absence of acriflavine). This could explain the correlation of "mutation fixation" with RNA synthesis as reported by Doudney and Haas (1959). Thus gene action (RNA synthesis on the DNA template) could uncover the UV-damaged site on the DNA, allowing its reaction (in a manner comparable to the damaged site - acriflavine interaction proposed) with some extra-DNA photoproduct, to produce that modification of the old DNA which leads to mutation. Of course, the hypothetical UV photoproduct would be present in the cell in too low a concentration to produce the lethal effect and effects on nucleic acid formation, as observed with added acriflavine.

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