NOTES

Effects of Acriflavine Orange on the Growth of Escherichia coli

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Received for publication 9 December 1971

Exposure of Escherichia coli to critical acridine orange (AO) concentrations did not result in loss of viability. However, the deoxyribonucleic acid (DNA) of cells exposed to such agents was rapidly degraded and repolymerized. On the other hand, a bacterium deficient in DNA repair (pol A+), lacking DNA polymerase) was sensitive to the action of AO. The DNA of such cells was also degraded but it was not repaired.

Although aminoacridine dyes can form complexes with deoxyribonucleic acid (DNA) in vitro (7,8), it is not known with certainty whether such complexes are also formed in vivo, nor whether, if they are formed, growth is affected. The present study is concerned with the effects of acridine orange (AO) on the growth of Escherichia coli and with an investigation of the properties of the DNA of cells exposed to such substances.

Exposure of $E$. coli W3110 thy- (pol A+) to low levels of AO ($\leq 60 \mu g/ml$) had little effect on growth. Higher concentrations ($\geq 100 \mu g/ml$), however, were bacteriostatic (Fig. 1). In contrast, $E$. coli p3478 (pol A-) (3) was rapidly killed by AO (Fig. 1). It is perhaps significant that this lethal effect was accompanied by an increase in the turbidity of the treated cultures which may be indicative of unbalanced growth (continued ribonucleic acid and protein synthesis in the absence of DNA production).

The presence of AO in cultures of pol A+ or pol A- strains resulted in the degradation of the existing (prelabeled) DNA to acid-soluble products (Fig. 2). In the case of the pol A+ strain we see the curious phenomenon of an extensive degradation of cellular DNA in the absence of bacterial death (Fig. 1 and 2). Analyses of the DNA species of these cells on alkaline sucrose gradients revealed (Fig. 3) that there was no significant difference in the sedimentation profiles of normal and AO-treated pol A+ cells. Contrariwise, the DNA derived from AO-treated pol A- bacteria was much smaller than normal DNA (Fig. 3), as evidenced by its very slow migration.

After prolonged (1 hr) exposure to 100 $\mu g$ of AO, the DNA of the AO-treated pol A- strain was very rapidly degraded and repolymerized (Fig. 3) but it was not possible to determine what form the AO-induced DNA polymers assume in the course of its repair or replication (Fig. 3).

Fig. 1. Effect of acridine orange (AO) on the growth of $E$. coli strains. Bacteria in medium HA (12) supplemented with thymine (5 $\mu g/ml$) were brought to the early exponential growth phase, at which time portions of the cultures were distributed into flasks containing premeasured amounts of AO. At intervals, portions from each culture were removed for enumeration of viable bacteria. Dilutions of these samples were spread onto agar containing medium HA and thymine (11). Symbols: $\bullet$, $\square$, $\bigcirc$, and $\blacktriangle$, bacteria exposed to 0, 20, 60, and 100 $\mu g$ of AO per ml, respectively.
AO/ml, pol A+ cells were still capable of incorporating 3H-thymidine into their DNA (Fig. 4), but this ability was almost completely lost by the pol A- cells.

Physical chemical and crystallographic studies have shown that aminoacridine dyes form complexes with purified DNA by intercalating between base pairs (7, 8). Aminoacridine dyes are mutagenic for bacteriophages, in which they cause frameshift mutations (2), but they are not (or only very weakly) mutagenic for bacteria (10). As a matter of fact, the low toxicity of aminoacridines for E. coli has permitted their use for "curing" cells of bacterial episomes (5; see also references 16 and 17).

The present study reveals that, when bacteria are exposed to AO, cellular DNA is degraded. Radiisotopic and light, as well as electron microscopic, data indicate (i) that major portions of the bacterial DNA are involved in this process and (ii) that the dye penetrates into the cell (unpublished data). In "normal" bacteria the DNA appears to be repaired immediately so that there is no discernible effect on the viability of the cultures (Fig. 1). Indeed, pol A+ cells exposed to AO incor-

**Fig. 2.** Effect of acridine orange (AO) on the degradation of cellular DNA. Bacteria were grown for several generations in the presence of 14C-thymidine. The cells were then washed and resuspended in fresh medium HA (12) containing thymine (5 µg/ml). Portions of the cultures were distributed into flasks containing premeasured amounts of AO. At intervals samples were removed for determination of radioactivity remaining acid-insoluble (13-15). Symbols: O and A, bacteria exposed to 0, 60, and 100 µg of AO per ml, respectively.

**Fig. 3.** Analysis on sucrose gradients of DNA from normal and acridine orange (AO)-treated cells. Bacteria [E. coli W3110 thy-pol A+ and E. coli p3478 pol A- (3)] in medium HA (12) containing thymine were supplemented with 3H-thymidine and uridine (386 µg/ml) (2). The bacteria were grown for several generations, harvested by centrifugation, resuspended in fresh medium, and aerated at 37 C for 30 min to exhaust the pool of radioactive precursor. Treated cultures were exposed to 100 µg of AO/ml for 90 min. Protoplasts were prepared (4) and deposited onto a linear (5-20%) sucrose gradient (pH 12) containing a layer of 0.1 ml of 0.5 N NaOH (9). After 15 min of standing at room temperature to insure complete lysing of the protoplasts, the materials were spun in the SW50 rotor of a Spinco model L-2 centrifuge for 6 hr at 30,000 rev/min. Fractions were then collected and processed for determination of radioactivity.
Fig. 4. Effect of acridine orange (AO) on incorporation of 3H-thymidine into DNA. Cultures during the early exponential phase of growth were distributed into flasks containing 3H-thymidine (0.5 µCi/ml), uridine (366 µg/ml), and various amounts of AO. At intervals the cultures were sampled for determination of the amount of 3H-thymidine incorporated into DNA (13-18). A, B, and C, bacteria containing 0, 60, and 100 µg of AO/ml, respectively.

ported 3H-thymidine under conditions which allowed repair synthesis to take place (Fig. 4). Furthermore, the alkaline sucrose gradient analyses reveal that this repair was complete, as evidenced by the superimposition of the sedimentation profiles of the DNA derived from normal and AO-treated pol A+ cells.

On the other hand, cells lacking DNA polymerase (pol A-) are more sensitive to AO, presumably because the repair process cannot be completed. [DNA polymerase has been implicated in the DNA repair process (3, 6).] Indeed, in the presence of AO such cells (i) cannot incorporate 3H-thymidine efficiently, and (ii) their DNA is considerably degraded. This degradation is irreversible, i.e., removal of AO leads neither to a repair of the DNA nor to a restoration of cell viability (unpublished data).

The fact that the DNA of seemingly “normal” cells exposed to acridines is degraded may explain some of the unusual biological effects of these dyes. Thus the synergistic effect of these substances with ultraviolet light might, in reality, be a reflection of the fact that the repair system of cells containing thymine dimers as well as acridine-induced damages may be overtaxed, as some of the same gene products are probably involved.

The most salient feature of the present study is, however, the finding that an intercalating agent affects the DNA of living cells and that the lesions so induced may be repaired without loss of viability.

We are grateful to John Cairns, Cold Spring Harbor Laboratory, for a gift of the bacterial strains used.

This investigation was supported by the Annie R. Masch Memorial Grant for Cancer Research from the American Cancer Society. One of the authors (H.S.R.) is a Research Career Development Awardee (2-K3-GM 29,024) of the U.S. Public Health Service, National Institute of General Medical Sciences.

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