Continuous-Flow System for Large-Scale Ultraviolet Irradiation of Bacterial Cells

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A quartz flow-cell system for irradiation of large volumes of *Escherichia coli* cultures with ultraviolet light is described. With this system kilogram quantities of irradiated cells can be obtained for biochemical studies. Changes in respiration and in specific activities of superoxide dismutase and catalase, after an ultraviolet treatment that reduced viability of culture samples to 0.2%, were in good agreement with those for cultures irradiated (52 J/m²) by a conventional small-scale method to produce the same reduction in viability.

Earlier work has shown that the death of *Escherichia coli* cells after ultraviolet (UV) irradiation is associated with cessation of respiration (7). Cells continued to respire for 1 h after UV irradiation, and after a relatively high fluence (52 J/m², 0.5% survival) cells stop respiring almost completely for several hours; a transitory cessation of respiration is seen when lower fluences are used. If individual cells stop respiring, they do so irreversibly and are dead (5, 8). Thus, resumption of respiration is due to surviving cells. Some evidence suggests that cessation of respiration is an induced response, in the sense of derepressing an operon (see reference 6 for summary); little is known of the sequence of biochemical steps that lead to this irreversible change. During the course of our studies of these biochemical changes, we were constantly pressed for the larger quantities of uniformly irradiated cells. Obviously, this required the scaling up of the customary "small-scale" procedure of growth, irradiation, subsequent aereration, and harvesting. Accordingly, we developed a quartz flow-cell system with which kilogram quantities of *E. coli* cells grown in a fermentor can be irradiated. The performances of such bacterial cells compared very favorably with those obtained by the standard small-scale procedure (3, 7).

**MATERIALS AND METHODS**

Growth, irradiation, subsequent incubation, and harvesting (large scale). *E. coli* B/r cultures (200 liters) were grown at 37°C in a 400-liter fermentor until the cell concentration reached 4 x 10⁸ cells/ml.

The cell suspension was then pumped through two flat, quartz flow cells in series. The flowing cell suspension was irradiated by two General Electric germicidal lamps, which were mounted 8.5 cm from the center of the cell above and below each flow cell (see description below). The flow rate through the quartz cells was 2.5 liters/min, and the concentration of cells being irradiated was kept constant by adjusting the flow rate of fresh medium into the fermentor tank. The survival of the irradiated samples was 0.1 to 0.5%, and the respiration of the same culture shut off at 60 min after UV irradiation for a period of several hours. These responses are characteristic of cultures irradiated (52 J/m²) using the small-scale method described earlier (7). The irradiated cell suspensions were collected in 40-liter insulated carboys and were aerated for 90 min by air under 5 pounds (ca. 2.27 kg) of pressure delivered through coarse, fritted-glass aerators at a rate of 12 to 15 liters/min. The cell suspensions were then pumped into a Sharples continuous-flow centrifuge rotating at 10,000 rpm. After being harvested, the cells were stored in 100-g samples at -80°C. This procedure provided a yield of nearly 1 kg of wet-weight cells in a 12-h working day.

Description of the quartz flow-cell irradiation system. Each flow-cell system was constructed from two 5- by 25-cm quartz plates and 1- by 0.5-cm Plexiglas strips (Fig. 1), which were sealed with epoxy cement so that the plates were 0.5 cm apart. To the open ends were attached two triangular plates, "inlet" and "outlet." The inlet was partitioned with three smaller strips, which caused turbulence of the incoming suspension so that it was rather uniformly irradiated. To the narrow ends of the inlet and outlet were glued Pyrex tubes (ID, 1.5 cm) to which were attached standard ball-and-socket ground joints (28/15).

The assembled flow cells were mounted on a stand supported by aluminum rods. A desk lamp holder with two 15-W, 15-inch (ca. 38-cm) General Electric germicidal lamps (G15T8) was mounted above and below each flow cell. Lamps were selected so that all had approximately the same output. The flow cells were both in the same plane and were 8.5 cm apart from the light source. A vertical cardboard baffle (not shown in the diagram) hung between the lamps and flow cells and insured that the cell suspension passing through each flow cell was irradiated only
by the lamps directly above and below it. The density of the suspension was 10^6 cells/ml. Since the light path of the cell suspension in the flow cell was only 0.5 cm and the suspension was irradiated equally from both sides, the fluence rate at the midpoint in the light path was approximately 20% of that at the inside surface of the flow cell.

**Respiration.** Oxygen consumption was followed with a Gilson differential respirometer.

**Viability.** Cell suspensions were diluted, and samples were spread on agar plates containing M63 glycerol medium solidified with 1.2% agar (Difco). The plates were incubated at 37°C, and colonies were counted after 2 days.

**Enzyme assays.** Superoxide dismutase (EC 1.15.1.1) was assayed according to the method of Marklund and Marklund (4) in which the rate of inhibition of autooxidation of pyrogallol by superoxide dismutase was followed. Catalase (EC 1.11.1.6) was assayed by following the change in absorption at 250 nm on a Beckman DB spectrophotometer attached to an automatic recorder (1).

**Chemicals.** Copper-zinc containing bovine superoxide dismutase was purchased from Truett Laboratories (Dallas, Tex.). Catalase was purchased from Worthington Biochemicals Corp. (Freehold, N.J.). All other reagents were analytical grade.

**RESULTS AND DISCUSSION**

In preliminary experiments involving irradiation of large volumes of *E. coli* suspensions, we varied the rate of flow through the quartz irradiation cell and the distance of the lamps from the flow cells. Respiration and viability measurements showed graduated responses. The final irradiation conditions gave the same responses as those using a fluence of 52 J/m^2 with the small-scale method, where more accurate dosimetry is possible. Figure 2 shows a typical respiratory response of a sample of irradiated cell suspension collected immediately after irradiation; the viability of this sample was 0.2%. We tested the respiration of cells sampled from the suspension aerated for 90 min and found that when aeration was inadequate the cells respired for 60 min before ceasing. With an air flow of 12 to 15 liters/min, we obtained cells that had shut off their respiration completely during the 90-min incubation period and that did not respire further. When an irradiated culture is shaken at 37°C in a nitrogen atmosphere for 90 min, the respiration pattern is nearly like that of one put in a respirometer flask immediately after UV exposure (3).

The requirement of oxygen for the shut off of respiration in irradiated cells suggested that oxygen was toxic, possibly as the result of accumulation of superoxide anions (2) and hydrogen peroxide. However, in a separate study, in which a conventional method of irradiation was used, we measured superoxide dismutase and catalase levels in cells incubated for 90 min after UV and found no evidence to support this notion (3). Table 1 shows the results of assays for the same enzymes in cells irradiated by the large-scale method described in this paper. The values, 6.3 and 4.2 units/mg of protein, respectively, are in good agreement with those (6.5 and 3.8 units/mg of protein, respectively) obtained in the small-scale irradiation study (3), where the respiratory and viability responses were the same as for the large-scale irradiated cells.

**TABLE 1. Effect of UV irradiation on the specific activities of catalase and superoxide dismutase in *E. coli* Br**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Units/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Catalase</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>5.9</td>
</tr>
<tr>
<td>Irradiated</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Irradiated cell suspensions were aerated at 37°C for 90 min before harvesting of cells. See text for details of large-scale irradiation methods.
These results give us confidence that we will be able to study biochemical changes in cells irradiated by the flow-cell method.

It is hoped that the type of quartz flow cell described here will have wider application for the exposure of cells with other types or radiations.

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LITERATURE CITED


