Inhibition of Oxygen Utilization and Destruction of Ubiquinone by Ultraviolet Irradiation of Thiobacillus thiooxidans

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Ultraviolet (UV) irradiation inhibited sulfur oxidation by cells of Thiobacillus thiooxidans. Sulfur-oxidizing activity decreased as the exposure time to UV light increased. A loss of the ability of cells to fix CO₂ paralleled the loss of sulfur-oxidizing activity. UV light photoinactivated ubiquinone purified from T. thiooxidans. The same percentage of sulfur-oxidizing activity and ubiquinone was destroyed after 15 min of UV exposure. Both the photoinactivation of sulfur oxidation and ubiquinone followed first-order reaction kinetics. The specific rate constants for both photoinactivations were nearly equal. Cells completely inactivated by UV light contained no ubiquinone. Ubiquinone was found to be a component of the cell wall-membrane complex.

The use of ultraviolet (UV) light to destroy bound quinones has been well established as a means of studying the role of these compounds in microbial metabolism (4). Brodie and Ballantine (5) reported that light with a wavelength of 360 mμ destroyed the ability of extracts of Mycobacterium phlei to oxidize various organic substrates because of the photoinactivation of a naphthoquinone. Other investigators (10) have found that short wavelength UV light (254 mμ) represses photosynthesis in chlorella because of the in vivo destruction of a plastoquinone.

Studies with membrane fragments of Micrococcus lysodeikticus (8) have shown that a cytochrome system is located in this cellular fraction. Irradiation of these fragments with near-UV light inhibited substrate oxidation, owing to the destruction of a membrane-bound naphthoquinone. Cook and Umbreit (6) reported that cells of the sulfur-oxidizing chemotrophic Thiobacillus thiooxidans contain important components of the cytochrome system, including a benzoquinone (coenzyme Q₈). In addition, it was earlier reported (1) that cell wall-membrane fragments of T. thiooxidans carried out a potassium cyanide-sensitive, sodium azide-sensitive oxidation of elemental sulfur. Thus, UV light may affect sulfur oxidation by T. thiooxidans and may provide a means for studying the role of ubiquinone in sulfur oxidation. This paper describes the effect of UV light both on ubiquinone and on the sulfur-oxidizing activity of T. thiooxidans.

MATERIALS AND METHODS

Organism. The organism used in these studies was the sulfur-oxidizing chemotrophic bacterium T. thiooxidans. It was originally obtained from R. L. Starkey, Rutgers, The State University, New Brunswick, N.J.

Preparation of cell-free fractions. T. thiooxidans was grown and harvested according to methods described earlier (1, 14). Cells were broken, whole cells were removed (1), and the broken cell suspension was centrifuged at 72,000 × g for 120 min. The pellet, which was considered the cell wall-membrane fraction, was freeze-dried and saved for later use. The soluble, supernatant fraction was stored by freezing at −20 C.

Extraction of ubiquinone. Ubiquinone-40 (coenzyme Q₈) was extracted from freeze-dried whole cells or freeze-dried cell wall-membrane fragments by rapid shaking in isooctane (2, 2, 4-trimethyl pentane) at room temperature (7). The soluble supernatant fraction was extracted with ether according to the method of Bishop and King (3). The ether extracts were combined and evaporated under nitrogen gas in a partial vacuum at room temperature. The residue was then dissolved in isooctane.

Purification of ubiquinone. Isooctane extracts containing ubiquinone were purified by column chroma-
Silicic acid and diatomaceous earth, in a ratio of 2:1, were slurried in isooctane and used to make a column (7 cm × 1 cm). All isooctane extracts were evaporated under nitrogen gas, in a partial vacuum, to a volume of about 2 ml and then placed on the column. Elutions were made with the following solvent combinations: isooctane (25 ml), 20% chloroform in isooctane (25 ml), and 50% chloroform in isooctane (25 ml). Ubiquinone was found in the 50% chloroform-isooctane fraction. This fraction was then evaporated and the residue was dissolved in 100% ethyl alcohol. Authentic ubiquinone-50 (Merck and Co., Inc., Rahway, N.J.) was also prepared for use by dissolving in 100% ethyl alcohol.

**Determination of ubiquinone concentration.** The concentration of ubiquinone was calculated in systems not irradiated by UV light by measuring the decrease in extinction at 275 μm when an ethyl alcohol solution was reduced with a few crystals of sodium borohydride. Calculations were based on a molar extinction coefficient of 1.47 × 10^4 (9). Spectrophotometry was done in a Beckman DB recording spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) by use of a 3-ml cuvette with a light path of 1 cm. The concentration of ethyl alcohol solutions of ubiquinone in irradiated systems was estimated by comparing the decrease in extinction at 275 μm with the decrease in extinction occurring after the complete photoinactivation of ubiquinone.

**Ultraviolet irradiation.** All materials were irradiated in shallow layers (3 to 5 mm) in small (5 cm) petri dishes. The small petri dishes were placed into larger (9 cm) petri dishes lined with aluminum foil to reflect light. The outer dishes were packed with ice to keep the materials cold during irradiation. A Mineralite (Ultra-Violet Products Inc., San Gabriel, Calif.), using a Sylvania 4-w germicidal lamp and fitted with a short-wavelength UV filter, was used as the light source (the principal wavelength was at 254 μm). Materials were irradiated by placing the light source directly above the 5-cm petri dish at a distance of 5 cm from the bottom of the dish.

**Respiration.** Oxygen absorption was measured in a standard Warburg apparatus at 28 C with shaking (140 oscillations per minute, amplitude at 4 cm).

**Carbon dioxide fixation.** The carbon dioxide-fixing capacity of cell suspensions irradiated for increasing periods of time was measured by adding sodium bicarbonate-14C to a side arm of each of the Warburg flasks, immediately after measuring sulfur oxidation. The manometer stopcocks were closed and the bicarbonate-14C was tipped into the cup of each flask. Incubation was allowed to proceed for 105 min with shaking. After this, unused bicarbonate-14C was removed by adding 0.3 ml of 5% HClO4 to the first side arm and 0.3 ml of 10% NaOH to the side arm of each dual side arm Warburg flask. The HClO4 was tipped into the reaction mixture. Unused bicarbonate-14C was absorbed by the NaOH. A portion of each cell suspension was removed from its respective flask and placed into an aluminum planchet. After drying, radioactivity was measured in a Tracerlab Multi-omatic automatic counter (Tracerlab, Richmond, Calif.).

**RESULTS**

**Extraction of ubiquinone.** Extraction of freeze-dried whole cells with isooctane yielded a substance which, after purification, had the typical oxidized spectrum (peak at 275 μm) and reduced spectrum (peak at 290 μm) of ubiquinone (Fig. 1).

**Effect of UV light on ubiquinone.** Exposure of purified ubiquinone to short-wavelength UV light for 15-min intervals caused the ubiquinone to be progressively destroyed (as shown by loss of the absorption spectrum) as the total time of irradiation increased (Fig. 2). As is illustrated in the graph, 50% of the original concentration of ubiquinone was lost after the first 15 min of irradiation. After 30 min of irradiation, 75% was lost, and after 45 min 93% of the ubiquinone was destroyed. At the end of 60 min of UV exposure, no ubiquinone could be detected. Similar results were obtained when an ethyl alcohol solution of authentic ubiquinone-50 was irradiated in the manner described above.

**Effect of UV light on sulfur oxidation.** Irradiation of a washed-cell suspension of *Thiothrix thiooxidans* with short-wavelength UV light caused the amount of sulfur oxidized to decrease as the exposure time to UV light increased (Fig. 3). Sulfur oxidation was monitored by oxygen absorption in a Warburg respirometer. In Fig. 3, the bars with cross-hatching indicate the amount of oxygen absorbed after each 15 min of UV

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Fig. 1. Spectra of ubiquinone purified from Thiothrix thiooxidans. Ubiquinone (8.8 × 10^-4 μmoles) was dissolved in 100% ethyl alcohol. The solid line represents oxidized ubiquinone. The dashed line represents reduced ubiquinone. Reduction was accomplished by adding a few crystals of sodium borohydride.
Effect of UV irradiation on T. thiooxidans

In the first 15 min, 49% of the sulfur-oxidizing activity was lost. An additional 15 min of exposure caused a loss of 76% of the activity. After 45 min of irradiation, a total of 82% of the sulfur-oxidizing activity was destroyed. In Fig. 4, the bars without cross-hatching show that carbon dioxide fixation decreased essentially in proportion to sulfur oxidation.

Table 1 presents the relationships between the ultraviolet photoinactivation of sulfur-oxidizing activity and the photoactivation of both ubiquinone-50 and ubiquinone isolated from T. thiooxidans. The data indicate that the decrease in sulfur-oxidizing activity is proportional to the photoactivation of ubiquinone after each 15 min of UV exposure.

Attempts to reverse the effects of UV light on sulfur oxidation, by adding ubiquinone purified from T. thiooxidans or ubiquinone-50, both treated according to the method of Beyer (2), were unsuccessful. The further addition of small amounts of reduced nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide phosphate, riboflavin phosphate, and flavin adenine dinucleotide also failed to cause any reactivation.

Effect of UV light on cellular ubiquinone. Small batches of whole cells of T. thiooxidans were irradiated with short-wavelength UV light for 60-min intervals. All of the cell suspensions were

![Graph showing optical density vs. wavelength](image-url)
tested for sulfur oxidation in a Warburg respirometer. If no sulfur was oxidized, the cells were freeze-dried and stored until enough accumulated (over 250 mg, dry weight) to be extracted. It was found that cells irradiated for 60 min contained no material with the characteristic absorption spectrum of ubiquinone.

Location of ubiquinone in T. thiooxidans. Inasmuch as sulfur oxidation by T. thiooxidans seems to be directly associated with the cell wall-membrane complex (1) and appears to be linked to a cytochrome system (1, 6, 13), ubiquinone should be located in the cell wall-membrane complex. In order to test this possibility, ubiquinone was extracted and purified from a known amount of freeze-dried whole cells. The amount of ubiquinone calculated from the decrease in extinction at 275 mµ, after reduction by a few crystals of sodium borohydride, was found to be 3.190 µmoles/g (dry weight) of whole cells. The concentration of ubiquinone in freeze-dried cell wall-membrane fragments was found to be 2.810 µmoles per amount of fragments derived from 1 g (dry weight) of whole cells. No ubiquinone could be detected in the soluble portion of the extract after the removal of the cell wall-membrane fragments. Ubiquinone, therefore, seems to be largely located in the cell wall-membrane complex.

Kinetics of the effect of UV light on sulfur oxidation and ubiquinone. If the logarithm of the amount of oxygen absorbed in 15 min by whole cells, after each time interval of UV exposure, is plotted against time, a straight-line function results (Fig. 4). When the logarithm of the concentration of ubiquinone from T. thiooxidans, found after each interval of UV exposure, is plotted against time, a straight line also results (Fig. 4). Both curves are characteristic of first-order reaction kinetics.

Calculation of the specific rate constants from the curves shown in Fig. 4 yielded the values presented in Table 2. The rate constants for each interval of exposure in a given system are nearly equal, indicating first-order reaction kinetics. The similarity between the rate constants obtained for the destruction of ubiquinone and

![Graph showing sulfur oxidation and ubiquinone data](http://jb.asm.org/)

**Fig. 4. Plots of the logarithms of the amounts of oxygen absorbed and of the concentration of ubiquinone, as a function of the time exposed to UV light. Both curves are based on data presented in Fig. 2 and 3.**

the rate constants obtained for sulfur-oxidizing activity provides a mathematical correlation between the two systems which strongly suggests that the destructive effect of UV light on sulfur oxidation is related to the destruction of cellular ubiquinone.

**DISCUSSION**

The data show that both the UV photoinactivation of the sulfur-oxidizing activity of cells of T. thiooxidans and the UV photoinactivation of purified ubiquinone follow first-

<table>
<thead>
<tr>
<th>System</th>
<th>Specific rate constants (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 to 15 min</td>
</tr>
<tr>
<td>Sulfur oxidation</td>
<td>4.65 × 10⁻²</td>
</tr>
<tr>
<td>Ubiquinone (T. thiooxidans)</td>
<td>4.83 × 10⁻²</td>
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* The rate constants were calculated from the curve shown in Fig. 4.
order reaction kinetics. The specific rate constants calculated from the inactivation curves were nearly equal for both systems. Approximately the same percentage of purified ubiquinone and sulfur-oxidizing activity was destroyed after each 15 min of UV exposure. These findings, as well as the fact that cells inactivated by UV light contain no detectable ubiquinone, appear to indicate that the inhibitory effect of UV light on sulfur oxidation may be due, in a large part, to the in vivo photoinactivation of ubiquinone. Thus, it seems that electron transport can be limiting in elemental sulfur oxidation.

Exposure of cells of *T. thioparae* to short-wavelength UV light also affected CO₂ fixation. As the exposure time increased, the amount of CO₂ fixed decreased nearly at the same rate as sulfur oxidation. This may be explained if the electrons produced from sulfur oxidation are used to provide chemical bond energy only by means of oxidative phosphorylation. This may indeed be the case since enzymes, such as adenosine-5'-phosphosulfate reductase, which are important in catalyzing the oxidation of thiosulfate to sulfate (12), with the production of high-energy phosphate bonds due to substrate phosphorylation, have not been found in *T. thioparae* (T. M. Cook, Ph.D. Thesis, Rutgers Univ., New Brunswick, N.J., 1963).

Ubiquinone, which is considered to be an important component of the bacterial cytochrome system, was found to be located entirely in the cell wall-membrane complex of *T. thioparae*. This agrees with the findings of other investigators (3, 11, 15) who state that quinones, along with other components of the cytochrome system, are exclusively located in the cell envelopes of both gram-negative and gram-positive bacteria.

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LITERATURE CITED