Inhibition of the Induced Formation of Tryptophanase in *Escherichia coli* by Near-Ultraviolet Radiation

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Induced formation of tryptophanase in *Escherichia coli* B/r is temporarily inhibited by near-ultraviolet (UV) irradiation. The inhibition is greater when irradiation is at 5°C than when at room temperature. Hence, the inhibition is the result of a photochemical, rather than photoenzymatic, alteration of some cellular component. The action spectrum has a peak in the region of 334 nm and is similar to that for growth delay. However, inhibition of tryptophanase formation is more sensitive to near-UV irradiation than to growth, respiration, and the induced formation of β-galactosidase. Thus, for tryptophanase the lack of formation cannot be due to general inhibition of metabolism. Pyridoxal phosphate absorbs in the near-UV region of the spectrum and is a cofactor for tryptophanase, but this enzyme in induced cells is not inactivated by near-UV-radiations. An experiment in which tolune-treated suspensions from irradiated and unirradiated cells were mixed showed that irradiation does not cause the formation of an inhibitor of tryptophanase activity. The possibility remains that the absorption of radiant energy by pyridoxal phosphate interferes with the synthesis of tryptophanase.

Near-ultraviolet (UV) and visible wavelengths are both effective in producing a photoreactivating effect in *Escherichia coli* B/r cells inactivated by far-UV radiations (2). While studying the effect of photoreactivation treatment on the induced formation of tryptophanase in this bacterium, we found that only visible wavelengths photoreactivate the system. On further examination, we found that near-UV radiations by themselves temporarily inactivate the tryptophanase-synthesizing system and that cells induced 30 min after the near-UV treatment do show a photoreactivation effect.

We report in this paper the action spectrum of the temporary inhibition of the induced formation of tryptophanase.

MATERIALS AND METHODS

*E. coli* B/r (ORNL), a radiation-resistant strain, was used in all experiments. The bacteria were grown aerobically in flasks swirling at 37°C. The M63 growth medium (11), which contained glycerol, was supplemented with 0.025% Casamino Acids. The cells were grown exponentially to a concentration of 4 × 10⁸ ml⁻¹. All experiments were started by using such cell suspensions.

Near-UV sources were General Electric F 15T8- BLB black lamps (emitting almost entirely in the peak near-UV region of the spectrum with a peak at 360 nm) and a quartz prism monochromator for monochromatic radiations. Irradiations, except where noted, were carried out at 5°C. For black-lamp irradiation, 20 ml of cell suspensions were irradiated for 20 min in 70-mm crystallizing dishes. The dose was 1.15 × 10⁶ ergs/mm² as measured with a meter described by Jagger (3). For the action spectrum, 2.1 ml of cell suspensions was irradiated in 1-cm³ spectrophotometer cuvettes. Transmittance depended on wavelength and varied from 28 to 43%. The incident dose rate was measured with a calibrated photocell, and the average dose rate through the sample was determined by the method of Morowitz (10). Wavelengths below 310 nm were filtered from the beam by a thin mylar film. During irradiation, the cuvettes were set in a three-sided copper jacket with an ice-filled reservoir. When not being irradiated, the cuvettes which contained irradiated cells stood in an ice-water bath. All cell suspensions were stirred continuously and were aerated every few minutes. Failure to aerate resulted in irregularities in the time-course curves for induced synthesis of tryptophanase.

Tryptophanase was induced by adding L-tryptophan (final concentration 500 μg/ml) to cells at 37°C. At intervals, 200-ml samples were blown into 0.1 ml of toluene, and the enzyme was assayed by the method of Pardee and Prestidge (12). In some cases, where noted, pyridoxal phosphate was omitted from the reaction mixture. The results are expressed as increases
(above the basal level) in tryptophanase content of a population of cells. One unit of enzyme activity is the amount that produces 1 amole of indole per min.

Respiration was measured on suspensions (2.0 ml of cells) by Warburg manometry (14). Growth was followed by measuring turbidity of the cultures at 650 nm; this method gives a measurement of the increase in cell mass. An optical density of 0.22 corresponds to a dry weight of 0.78 mg/ml.

RESULTS AND DISCUSSION

Figure 1 shows that black-light treatment for 20 min at 5 C inhibits the induced formation of tryptophanase by about 90% for approximately 20 min. The experiment was carried out at 5 C for two reasons: first, to provide conditions such that the unirradiated cells, after being held in the dark during the 20-min period when other cells were irradiated, would synthesize enzyme at the same rate as those induced before being held; second, to determine whether the inhibitory action is caused by photochemical or photoenzymatic reactions. When black light is given at room temperature, inhibition is less than with irradiation at 5 C. Therefore, we conclude that the inhibition is the result of photochemical damage from which the cell recovers. The β-galactosidase-synthesizing system is affected much less by near UV at 5 C than is the tryptophanase system. There is only a 2- to 3-min delay in synthesis, and the differential rate 5 min after irradiation is about the same as that for the unirradiated cells (data not shown). Near-UV radiations are very effective for photoreactivation of this system after partial inhibition by far-UV radiations. However, after far-UV irradiation, the black light produces a dual effect on the tryptophanase system, photoreactivation, and temporary inhibition. The survival of colony-forming ability after black-light treatment alone is 100%.

We investigated the possibility that the inhibition of the induced formation of tryptophanase by near UV (Fig. 1) is caused by catabolite repression, as is the case for β-galactosidase by far-UV irradiation (13). However, even though the presence of glycerol results in catabolite repression, the inhibition of tryptophanase formation is independent of catabolite repression (manuscript in preparation). The irradiation inhibits enzyme formation by about the same factor whether or not glycerol is in the incubation medium after induction.

Action spectrum studies were carried out to determine the nature of the absorption spectrum of the photolabile cellular component responsible for the inhibition of induced tryptophanase formation. Four wavelengths of monochromatic radiation were used: 313, 334, 365, and 405 nm. The kinetics for induced tryptophanase formation after various doses of 334-nm radiation are shown in Fig. 2. Sets of curves similar to those of Fig. 2 were obtained with larger doses at 313 and 365 nm. No inhibition was obtained with 405-nm light after doses up to 4.3 × 10^{6} ergs/mm{2}. Data for dose-response curves (Fig. 3) were obtained by reading, from the kinetic curves, the amounts of enzyme formed at 20 min after induction. The relative efficiencies of these wavelengths were obtained by taking first the reciprocal of the interpolated 1/e doses (the dose that reduces tryptophanase formation to 37% of that of the unirradiated cells). The reciprocal value for the most efficient wavelength was set at 100; all others were normalized to it, and the relative values, corrected for quantum energy, were plotted as a function of wavelength (Fig. 4). The resulting action spectrum has a peak in the region of 334 nm; the curve falls off rapidly toward the long end of the spectrum.

**Fig. 1.** Inhibitory action of black light (BL) at 5 C on the induced formation of tryptophanase in E. coli B/r. The BL dose was 1.15 × 10^{6} ergs/mm{2}.

**Fig. 2.** Inhibitory effect of various doses of 334-nm radiation on induced synthesis of tryptophanase.
The action spectrum for the inhibition of tryptophanase matches very well with the action spectra for photoprotection (4) and growth delay (5) in *E. coli* B (see Fig. 4). We were interested in the possibility that a photochemical change in one type of compound is responsible for all three effects. Jagger (1), on the basis of 104 ergs/mm² work by Kashket and Brodie (6, 7), considers quinones such as benzoquinone in the electron transport system to be the likely chromophores for growth delay and photoprotection. In Fig. 5, we compare the effects of two doses of 334-nm radiation on the processes of tryptophanase synthesis, growth, and respiration. Growth and respiration are affected about equally, but the tryptophanase-synthesizing system especially at the higher dose is more sensitive than the other.

**Fig. 3.** Dose-response curves for four wavelengths on the induced formation of tryptophanase. Data for the 334-nm curve were taken from Fig. 2.

**Fig. 4.** Action spectrum for inhibition of the induced formation of tryptophanase by near-UV radiations. The relative efficiencies of each wavelength are indicated by circles on a graph from Jagger et al. (5). The solid and dotted lines are the action spectra for photoprotection and growth delay, respectively.

**Fig. 5.** Inhibitory effects of two doses at 334-nm radiations on three cellular processes. (a) Respiration; (b) growth; and (c) induced synthesis of tryptophanase. For the respiration experiment, 10 min of thermal equilibration at 37 °C was required before readings were taken.
two processes. The difference in sensitivity is best seen when the data in Fig. 5 for tryptophanase synthesis and growth are used in a differential plot (Fig. 6). Similar results were obtained with black-light irradiation. Although 334-nm radiations and black light cause only a small and about equal inhibitory effect on respiration and growth, they cause a relatively large effect on the induced synthesis of tryptophanase. Studies on the effects of 334-nm and black light illumination on the kinetics of deoxyribonucleic acid, ribonucleic acid, and protein synthesis (as indicated by incorporation of tritiated precursors) give qualitatively the same information as the kinetic studies of respiration and growth. There was little effect on these parameters at doses that have a big effect on the induced formation of tryptophanase. As with the black-lamp experiments, cell survival was 100% after the largest dose of 334-nm illumination.

The differential plot for growth and the tryptophanase formation, together with the respiration data, make it unlikely that the inhibition of tryptophanase formation is linked to the inhibition by UV of either of the other two processes. After 20 min of irradiation at 334 nm, growth is reduced to about 30% and tryptophanase to 5% of the control values—a sixfold difference in activities remaining. A comparison of the absolute values of the action spectra for tryptophanase inhibition and for growth delay (S) gives the same information; although both spectra have their maximum at the same wavelength, the 1/e doses for tryptophanase inhibition are only about 10% of those for growth delay. The lack of

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### Table 1. Test for inhibition of tryptophanase by toluene-treated suspensions of irradiated (334 nm) cells

<table>
<thead>
<tr>
<th>Nonirradiated/irradiated toluene-treated suspensions</th>
<th>Enzyme activity (units/ml)</th>
<th>Expected value^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:0</td>
<td>0.54 X 10^-3</td>
<td>0.38</td>
</tr>
<tr>
<td>2:1</td>
<td>0.43 X 10^-3</td>
<td>0.30</td>
</tr>
<tr>
<td>1:1</td>
<td>0.33 X 10^-3</td>
<td>0.22</td>
</tr>
<tr>
<td>1:2</td>
<td>0.25 X 10^-3</td>
<td></td>
</tr>
<tr>
<td>0:2</td>
<td>0.07 X 10^-3</td>
<td></td>
</tr>
</tbody>
</table>

^a Unirradiated and irradiated cells (1.2 X 10^4 ergs/mm^2) were induced for 20 min and treated with toluene. The suspensions were mixed in the proportions shown, and 0.2 ml of each mixture was assayed for tryptophanase activity. Pyridoxal phosphate was not added to the reaction mixture.

^b If no inhibitor were present.

### Table 2. Effect of 334-nm irradiation on the activity of tryptophanase in induced cells

<table>
<thead>
<tr>
<th>Min of incubation at 37 C</th>
<th>Enzyme activity (units per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irradiated</td>
</tr>
<tr>
<td>0</td>
<td>1.15 X 10^-3</td>
</tr>
<tr>
<td>10</td>
<td>1.23 X 10^-3</td>
</tr>
<tr>
<td>30</td>
<td>1.15 X 10^-3</td>
</tr>
<tr>
<td>40</td>
<td>1.23 X 10^-3</td>
</tr>
<tr>
<td>50</td>
<td>1.27 X 10^-3</td>
</tr>
<tr>
<td>60</td>
<td>1.28 X 10^-3</td>
</tr>
</tbody>
</table>

^a Cells were induced and incubated at 37 C for 1 hr, after which they were washed free of inducer and suspended in growth medium at the same concentration as at the time of induction. A 2-ml amount of the suspension was irradiated with a dose of 1.2 X 10^4 ergs/mm^2. The irradiated and nonirradiated samples were then incubated at 37 C, and samples were assayed at intervals for tryptophanase activity. Pyridoxal phosphate was not added to the reaction mixture.

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![Graph](http://jb.asm.org/)

FIG. 6. Differential plot showing increase in enzyme as a function of increase in cell mass as measured by turbidity changes. The data were taken from Fig. 5.
are present (8). Of some interest is the fact that addition of cysteine results in shift of both peaks to the shorter wavelengths with the main (longest wavelength) peak being at about 335 nm. The possibility was considered that the near-UV radiation might interact with pyridoxal phosphate, or with some other cell constituent, and that the resulting photoproduc might interfere with the activity of the tryptophanase. To test this idea, an experiment was carried out in which cells were induced for 20 min and toluene-treated suspensions of irradiated and unirradiated cells were mixed in various proportions before assaying. For this experiment and the one to follow, pyridoxal phosphate was omitted from the assay mixture, and we depended on the cofactor in the cells to make the reaction take place. The amount of enzyme activity was proportional to the amount of each suspension present, and the toluene-treated suspensions from irradiated cells had no effect on the enzyme activity of those from unirradiated cells (Table 1).

The absorption spectrum of holotryptophanase has maxima at 337 and 420 nm (9). We induced cells to make tryptophanase and, after washing the cells to get rid of tryptophan, irradiated them at 334 nm. We then looked for changes in enzyme activity at various times after irradiation. Little change in enzyme activity was caused by 334-nm irradiation or by subsequent incubation at 37 C (Table 2). This result indicates that photochemical destruction of pyridoxal phosphate is not the cause of the lack of appearance of tryptophanase activity in near-UV irradiated cells. The result does not rule out the possibility that the absorbance of radiant energy by pyridoxal phosphate interferes with the synthesis of tryptophanase.

It seems probable that the photoinhibition of the induced synthesis of tryptophanase involves the direct photochemical inactivation of some cellular component because, as in the case of photoprotection (2), near-UV radiations are effective at low temperatures. The action spectrum is our only clue to the characteristics of the photochemical target. Two questions whose answers await the identification of this substance are: (i) What is the role of the undamaged target material in the synthesis of tryptophanase? (ii) Is the target material inactivated irreversibly so that new synthesis of the substance is necessary, or is there a cellular repair process which restores its chemical activity?

ACKNOWLEDGMENT

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LITERATURE CITED