JOURNAL OF BACTERIOLOGY, Jan. 1976, p. 111-118
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Vol. 125, No. 1
Printed in U.S.A.

Inactivation of Adenosine 5'-Triphosphate Synthesis and Reduced-Form Nicotinamide Adenine Dinucleotide Dehydrogenase Activity in *Escherichia coli* by Near-Ultraviolet and Violet Radiations

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Received for publication 4 August 1975

Near-ultraviolet (near-UV) light (300 to 380 nm) is a significant component of sunlight and has a variety of effects on biological systems. The present work is an attempt to identify chromophores (molecular absorbers of light) and targets (critical damaged molecules) for inhibition of adenosine triphosphate (ATP) synthesis in *Escherichia coli* by near UV. The fluence at 334 nm required for 37% survival of net ATP synthesis (F_{37}) in *E. coli* AB2463 in succinate medium is 140 kJ/m^2. The action spectrum for this inactivation is almost structureless, exhibiting a smooth transition from high efficiency at 313 nm to low efficiency at 405 nm. The action spectrum for inhibition of net ATP synthesis is consistent with the chromophore being either ubiquinone Q-8 or vitamin K_2. The fluence required is consistent with ubiquinone Q-8 also being a target molecule. The activity of reduced nicotinamide adenine dinucleotide dehydrogenase in extracts of *E. coli* B is also inactivated by near UV and shows an F_{37} of about 40 kJ/m^2. The action spectrum for this effect is quite structureless; it shows high efficiency at 313 nm and low efficiency at 435 nm. The data do not suggest a target molecule for this action, although it is possible that ubiquinone Q-8 absorbs the near-UV energy and then passes it on to some other target molecule. The data further indicate that inactivation of the oxidative phosphorylation system is not a primary factor in near-UV-induced growth delay in *E. coli*.

Near-ultraviolet (near-UV) light (300 to 380 nm), an important component of sunlight, has a variety of effects on biological systems (10). Although the fluences (doses; see reference 23) required for these effects are relatively high compared to those required in the far-UV region (below 300 nm), they may be easily produced by relatively short exposures to sunlight; for example, a 9-min exposure to unfiltered sunlight can produce 90% inhibition of bacterial growth (11). It is therefore important to estimate the sensitivities of various biological systems to near-UV damage and to attempt to determine the molecular targets for these damages.

It was shown some time ago by Brodie and Ballantine (7) that oxidative phosphorylation in extracts of *Mycobacterium phlei* is quite sensitive to 360-nm light. Those studies showed that phosphorylation was considerably more sensitive than oxidation. Later studies (15) showed that continuous illumination of *Escherichia coli* W with black light (broad-band near UV) produced a slightly lower growth rate when the cells were incubated in glucose, but almost complete cessation of growth was produced when the cells were incubated in succinate. These results were interpreted to mean that growth inhibition in *E. coli* was probably caused by damage to the oxidative phosphorylation system and, in particular, to the isoprenoid quinones, which are the most near-UV-labile components of the electron transport system. Subsequent work in our laboratory (10) was conducted in an effort to demonstrate that this was indeed so. These experiments have, however, proved inconclusive. In particular, a recent study (25) shows that ubiquinone Q-8 is much too resistant in vivo to be a target for near-UV-induced growth delay. More recent studies in our laboratory indicate that the primary chromophore for near-UV-induced growth delay in *E. coli* is probably 4-thiouracil in transfer ribonucleic acid (21; T. V. Ramabhadran and J. Jagger, Proc. Natl. Acad. Sci., U.S.A., in press).

Nevertheless, oxidative phosphorylation is

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relatively sensitive to near UV and may be involved in a wide variety of other near-UV effects. It has recently been found, for example, that light of wavelengths above 400 nm inactivates several active transport systems in *E. coli* (2). It is likely that light of wavelengths below 400 nm would also be effective on these systems, and that adenosine triphosphate (ATP) synthesis would be intimately involved. Preliminary reports show that membrane transport of sugars (A. L. Koch, R. J. Doyle, and H. E. Kubitschek, personal communication) and amino acids (G. D. Sprott, W. G. Martin, and H. Schneider, personal communication) in *E. coli* is greatly reduced by near-UV radiation.

The present study was conducted in an effort to determine the sensitivity of *E. coli* cells to near-UV inhibition of net ATP synthesis under oxidative conditions and to determine the action spectrum for this effect, with the goal of identifying chromophores and targets. *E. coli* strain AB2463, a *recA* strain, was used because it is capable of extensive photoprotection (17), in the hope (subsequently abandoned because of our recent findings with transfer ribonucleic acid; see above) that these studies might cast some light on the photoprotection phenomenon, which is believed to operate through the induction of a growth delay (10).

Similar studies were also conducted on reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase inactivation in extracts of *E. coli* B.

Our results suggest that isoprenoid quinones may be involved as chromophores or targets in these effects. They also indicate that inactivation of oxidative phosphorylation is not a primary factor in near-UV-induced growth delay in *E. coli*.

MATERIALS AND METHODS

Cell cultures. *E. coli* AB2463 (*recA13*), a mutant of *E. coli* K-12 requiring Thr, Leu, Pro, His, Arg, and thiamine for growth (1), was used for most of the ATP studies. A stationary-phase cell suspension was diluted 1:20 in M9 mineral salts medium (18) without glucose ($M9^-$), supplemented with 0.5% sodium succinate and 0.25% Casamino Acids (final concentrations), and grown in a 37 C water bath shaker for approximately 2.5 h to the late logarithmic growth phase (2×10^8 cells/ml). The cells were harvested by centrifugation, washed twice with $M9^-$, and starved in $M9^-$ at 37 C for 2 h. These cells were used for irradiation.

The NADH studies were done with *E. coli* B (Harm), which was grown in M9 medium (containing glucose) supplemented with 0.25% Casamino Acids.

A few experiments were done with *E. coli* K-12.

Viable cell counts were determined by counting colonies on nutrient agar plates after 24 h of incubation at 37 C.

Radiation treatments. Cells were irradiated at concentrations of about 2.0×10^8 cells/ml in a Pyrex spectrophotometer cuvette placed in the exit beam of a Bausch and Lomb 33-86-45-49 grating monochromator equipped with a Philips SP-500 high-pressure mercury-vapor arc lamp. Both irradiated samples and dark controls were stirred continuously during irradiation. A Mylar filter that transmits <1% at 305 nm screened out short wavelengths that might pass the monochromator. Fluences incident on the cuvette were measured with a calibrated Eppley thermopile. The cultures to be irradiated showed an optical density of 0.70 at 334 nm for a 1-cm path length on a Zeiss PMQ II spectrophotometer. Much of this measured optical density is due to light scattering. We estimate a correction factor of 0.90 for the average fluence rate at 334 nm encountered by cells in the suspension (12). This, times a transmission factor of 0.96 for one wall of the cuvette, gives a net correction factor of 0.86. All fluences reported herein are so corrected.

Some of the NADH experiments involved irradiation by General Electric 15-W black-light lamps (T8 BLB).

ATP synthesis. Samples were drawn from each irradiated suspension, mixed with prewarmed 2% (final concentration) sodium succinate in $M9^-$, and shaken in a water bath shaker for times up to 90 min. Samples were removed from the water bath shaker at various times and mixed immediately with cold $HClO_4$ (see below). An unirradiated control was run at the same time.

For some experiments, succinate was replaced by sodium pyruvate (0.1%) or glucose (0.1%).

Extraction of ATP. ATP extraction from cells was carried out by the procedure of Ramirez and Smith (22). A 0.30-ml cell sample (irradiated or control) was mixed with 0.10 ml of 17.5% (wt/vol) cold perchloric acid ($HClO_4$). The mixture was then either kept for 1.0 h at room temperature or stored at 4 C overnight. A small drop of phenol red was added to the mixture as a pH indicator prior to neutralization of the $HClO_4$ with 0.10 ml of an alkaline buffer [17.5 ml of saturated KOH, 22.5 ml of 1 M KCl, and 60 ml of 0.1 M tris(hydroxymethyl)aminomethane]. The precipitate was removed by centrifugation at $12,000 \times g$ for 20 min in a refrigerated (2 C) Sorvall centrifuge. The clear pinkish solution was decanted into a tube, which was subsequently kept in crushed ice.

Determination of ATP. The assay of McElroy and Seliger (20) was used. Firefly tail extracts in 50-mg vials (which contain both luciferin and luciferase) were purchased from Sigma Chemical Co., St. Louis, Mo. The contents of one vial was suspended in 5.0 ml of water, centrifuged, and filtered through a cotton plug.

A fraction (0.30 ml) of the unknown sample was added to 1.00 ml of a Mg buffer (0.050 M glycyl-glycine buffer, pH 7.4; 0.010 M $MgCl_2$) in a 3-ml glass cuvette. The cuvette was placed in a dark chamber in

front of the large end-window photomultiplier of an Aminco-Chance Duochromator, the output of which was connected to a Honeywell Electronik-19 recorder running at a speed of 40 s/inch (2.5 cm). When 0.20 ml of the firefly tail extract was injected into the sample (Hamilton hypodermic syringe with Chaney adaptor), a light flash occurred, which rapidly decayed (in ~15 s) to a value about one-third the peak height and then decayed slowly (1 to 3 h) to zero. The intensity of the light 30 s after injection is directly proportional to ATP concentration. Arbitrary intensity units (recorder deflections) were used for either relative or absolute measurements of ATP concentration. We find that an average cell of *E. coli* in logarithmic growth in M9 medium contains approximately 1.7×10^{-10} μg of ATP. This value is in good agreement with values quoted in the literature (e.g, 1.45×10^{-10} μg/cell for *E. coli*, in all growth phases, grown in nutrient broth and sonicated; A. J. D'Eustachio, and G. V. Levin, Bacteriol. Proc., p. 121, P119, 1967).

NADH dehydrogenase assay. One liter of *E. coli* B cells was grown aerobically to stationary phase in M9 plus Casamino Acids and harvested by centrifugation. The cell pellet was washed two times in 0.067 M phosphate buffer and resuspended in 5 ml of 0.05 M glycyl-glycine buffer, pH 8.0. This suspension was sonicated (Sonifier cell disruptor, Heat Systems Co., Melville, N.Y.) with three 15-s pulses at 120 W. The sonic extract was centrifuged for 20 min at 27,000 × *g*, and the pellet was discarded. The extract was diluted twofold. One milliliter of this solution was irradiated by light of the desired wavelength, as described above. The extract had an optical density less than 0.5 at 334 nm, and a correction was made, as above, for the lowered average fluence in the extract.

The NADH dehydrogenase activity of a given solution was determined by measuring the rate of decrease in optical density at 340 nm in an Aminco-Chance recording spectrophotometer (Duochromator). The reaction was started by adding 0.1 ml of NADH (17 μg) to a glass spectrophotometer cuvette containing 2.1 ml of 0.05 M glycyl-glycine buffer (pH 8.0), 0.1 ml of 0.01 M $MgCl_2$, and 0.2 ml of cell extract (110 μg of protein, determined by the method of Lowry et al. [19]).

Whole cells of *E. coli* B were also irradiated by four black-light lamps for 1.0 h, at a source-to-sample distance of 14 cm. After irradiation, crude extracts were prepared, and the NADH dehydrogenase activity was assayed by the procedures described above. Unirradiated controls were also run.

RESULTS

ATP synthesis. Experiments were performed to ensure that our measurements reflected real changes in ATP content per cell. Figure 1 shows that the ATP level drops slowly during starvation and then rises abruptly upon addition of succinate (without Casamino Acids), followed by a plateau. The population level increases by 60% during starvation, drops abruptly upon addition of succinate, and then quickly regains the level shown immediately before succinate addition. We refrain from speculation on the cause of these changes, but note that (i) throughout the entire incubation period, the average viable cell count remains within 30% of the mean value for the entire period, and (ii) the drop in viable cell count upon addition of succinate would make the change in ATP content per viable cell even greater than the change in ATP content shown in Fig. 1 for the suspension (the latter is used in all later calculations). These considerations indicate to us that the rise in ATP content during the first 15 min of incubation in succinate represents a real increase in ATP content per cell and is thus a measure of the efficiency of the ATP synthetic machinery.

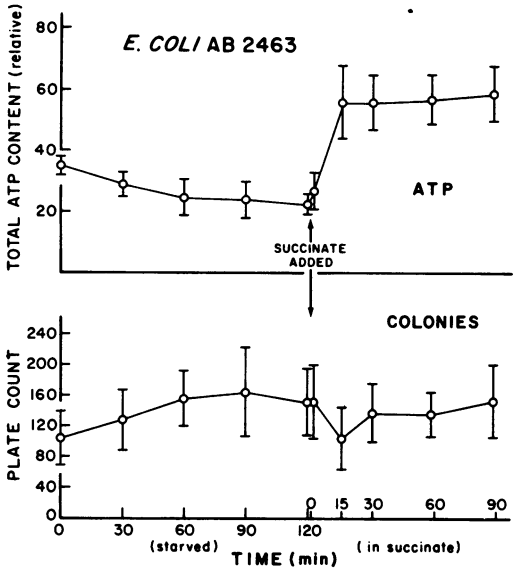

FIG. 1. *ATP content (upper) and viable cell count (lower) of suspensions of E. coli AB2463 during starvation and subsequent incubation in succinate. Logarithmic-phase cells were washed and then incubated (starved) in M9⁻ at 37 C in a water bath shaker for 2 h (lefthand side of graph). Sodium succinate was then added (final concentration, 2%), and the cells were incubated for another 90 min (right side of graph). Levels during succinate incubation have been corrected for the dilution of the population to 60% of its original concentration (mimicking operations necessary during irradiation experiments). Average values and standard deviations are shown for four experiments.*

Figure 2 shows the depression of ATP synthesis caused by various fluences of 334-nm light. Both the plateau level of ATP concentration and the initial rate of synthesis are affected by the light. For reasons of convenience and higher precision, we chose to measure the depression of ATP synthetic ability by the lowering of the pla-

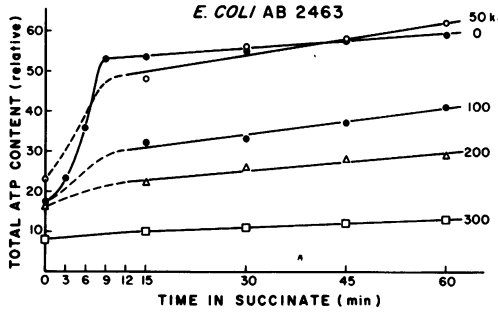

FIG. 2. *Depression of net ATP synthesis in E. coli AB2463 by various fluences of 334-nm light (numbers at right of curves, in kJ/m²). A small volume (1.0 ml) of starved cell suspension was irradiated and then mixed with 0.7 ml of M9⁻ supplemented with 2% succinate (final concentration) and placed in a 37 C water bath shaker. Samples of 0.30 ml were withdrawn at various times and assayed for ATP content. Cells were treated otherwise as in Fig. 1.*

teau level. Since samples had to be irradiated sequentially, the initial ATP concentration (zero time) drifted somewhat (±50%). In analyzing these data, therefore, the curves were displaced (by an additive factor) to the same initial ATP level, and experiments were done in reverse sequence on alternate days in an effort to average out these systematic variations. As a measure of net synthesis of ATP, we used the average of the observed values at 15, 30, 45, and 60 min minus the zero value (just before addition of succinate).

From data such as those shown in Fig. 2, one may calculate the depression of ATP level as a function of radiation fluence. This is presented in Fig. 3 for four different wavelengths of light. The data points are well fitted by straight lines (exponential loss), but all wavelengths show a threshold fluence, which increases with wavelength. The data for 313 nm and 334 nm are identical within experimental error. At 334 nm, the value of the threshold fluence is about 40 kJ/m², and the value for 37% survival of ATP synthetic ability (F_{37}), including the threshold fluence, is about 140 kJ/m².

The curves of Fig. 3 are quite similar, in the sense that multiplication of the fluences for each curve by a constant factor causes the curves to overlap reasonably well. This indicates that the same mechanism for inhibition of ATP synthesis is operating at all four wavelengths. At a level of 70% survival of ATP synthetic ability, the percentage of survival of colony-forming ability is about 80% at 405, 366, and 334 nm and 1% at 313 nm. This indicates that killing does not affect the ATP synthetic ability, as one would suspect, since killing is a measure of cell division, which is observed at a much later time. The data, however, are consistent with the idea that inhibition of ATP synthesis may play a role in cell death at 334-nm and longer wavelengths.

The data of Fig. 3 are plotted as an action spectrum (closed circles) in Fig. 5, where each relative efficiency represents the factor by which each curve in Fig. 3 would have to be multiplied to make it similar to the curve at 313 nm. This is tantamount to including the threshold; however, an action spectrum based on the relative slopes of the curves in Fig. 3 differs only slightly from the one we show. The point at 334 nm in Fig. 5 falls below that at 313 nm because of the quantum correction.

Table 1 shows the effect of 334-nm radiation on net ATP synthesis in the presence of different substrates. For strain AB2463, ATP synthesis in succinate shows the threshold apparent in Fig. 2 and 3. The K-12 parent strain does not show this threshold, although the final sensitivity is about the same. Strain AB2463 shows no depression of ATP synthesis in the presence of glucose or pyruvate; in fact, it shows a slight enhancement of the ATP level. This behavior is not exhibited by the K-12 parent strain, but net ATP synthesis in strain K-12 is only about one-third as sensitive in glucose as in succinate.

The slight rise in ATP level in glucose and

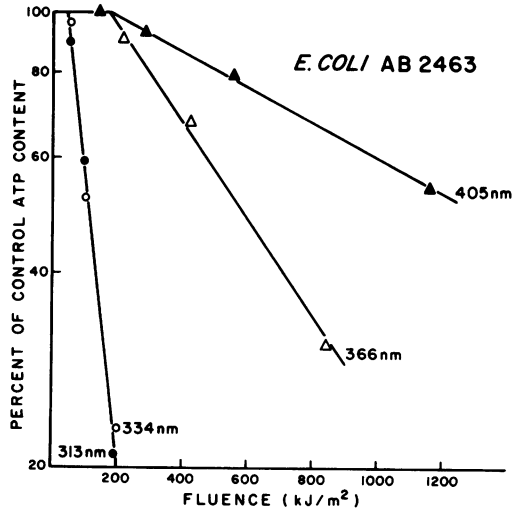

FIG. 3. *Action of different wavelengths of light (shown on curves) on net ATP synthesis in E. coli AB2463. The percentage of drop in plateau level from data such as are shown in Fig. 2 is plotted on a logarithmic scale as a function of radiation fluence. Average of two experiments at each wavelength.*

TABLE 1. *Effect of 334-nm radiation on net synthesis of ATP in E. coli K-12 strains growing on different substrates*[a]

Fluence (kJ/m²)	Substrate	% of control	
		K-12 AB2463	K-12
0	Succinate (2%)	100	100
50		103	70
100		57	51
150		34	
200		21	16
0	Glucose (0.1%)	100	100
50			98
100			86
200		122	66
0	Pyruvate (0.1%)	100	
200		118	

[a] Typical single experiments.

pyruvate after irradiation of strain AB2463 (seen in many experiments) might be due to a disturbance of equilibrium. Since we are simply measuring the ATP content at a given time, this represents the amount of ATP synthesized minus the amount of ATP utilized (net ATP synthesis). If the rate of utilization were to decrease greatly (due perhaps to radiation damage), then net ATP could rise even though there were concomitant deleterious effects on ATP synthesis. However, we find that addition of 10^{-4} M cyanide or dinitrophenol to the system after irradiation results in no net ATP synthesis (neither a rise nor a fall). Thus, it would appear that the rate of utilization of ATP under our conditions is low compared with the rate of synthesis. Therefore, a decreased rate of utilization does not seem to explain this "stimulation" effect.

NADH dehydrogenase. Figure 4 shows the loss of NADH dehydrogenase activity that results from irradiation of extracts of *E. coli* B at different wavelengths. It can be seen that radiation at 313 nm is most effective (at 334 nm, the F_{37} is 40 kJ/m²).

The action spectrum for this loss of NADH dehydrogenase activity (relative negative slopes of curves in Fig. 4, with quantum correction) is plotted in Fig. 5 (closed triangles).

Bragg (3) has recently shown that near-UV irradiation of a particulate fraction of *E. coli* inhibits NADH oxidase activity (measured both spectrophotometrically and by O_2 uptake). We find that NADH dehydrogenase activity in extracts is rapidly lowered even if whole cells are irradiated, with a sensitivity roughly the same as for extracts.

Relative sensitivities of various near-UV effects. Table 2 compares the F_{37} values at 334 nm for various effects of near UV. Growth inhibition is the most sensitive phenomenon studied, and photoalteration of ubiquinone Q-8 in vivo is the most resistant.

DISCUSSION

The early work of Kashket and Brodie (16) on membrane fractions of *E. coli* W showed that oxidation of NADH by a cytochrome acceptor can be inactivated by near UV and restored only by addition of unirradiated vitamin K_2, whereas electron transport from succinate to a cytochrome acceptor, also inactivated by near UV, is restored only by addition of ubiquinone. They suggested, therefore, that vitamin K is an essential link in electron transport in *E. coli* between the NADH-dehydrogenase-flavoprotein complex and cytochrome b_1, and that ubiquinone Q-8 performs a similar function, associated with the succinic-dehydrogenase-flavoprotein complex. This scheme, however, has not been confirmed in other strains of *E.*

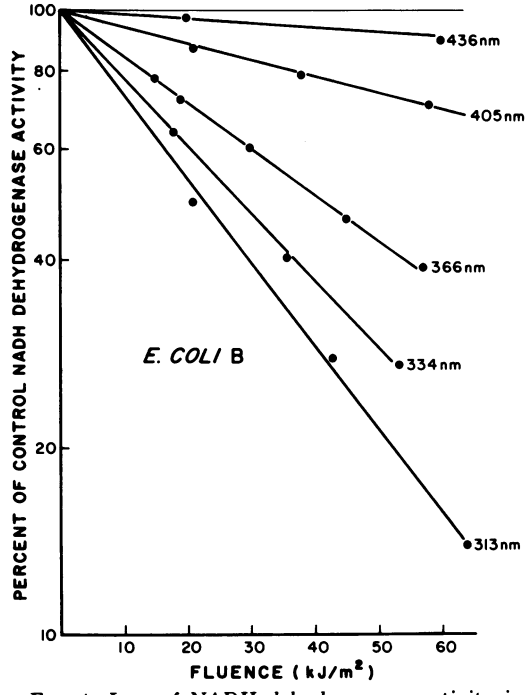

FIG. 4. *Loss of NADH dehydrogenase activity in extracts of E. coli B as a function of fluence at various wavelengths. Assay is based upon rate of loss of NADH absorbance at 340 nm. Average of two experiments at each wavelength.*

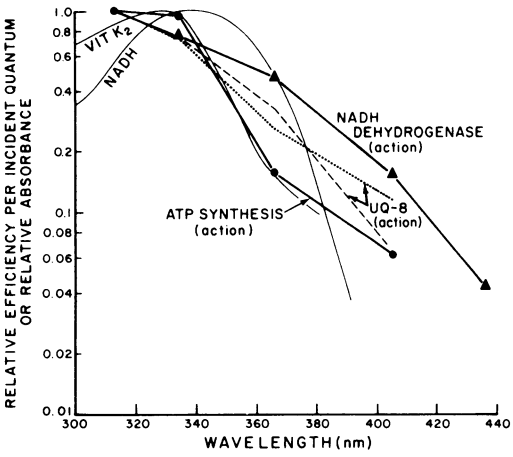

FIG. 5. *Action spectra (heavy solid lines) for (●) inhibition of net ATP synthesis in E. coli AB2463 and (▲) loss of NADH dehydrogenase activity in E. coli B. Shown for reference are the action spectra for inactivation of ubiquinone Q-8 (dashed line) in E. coli B and (dotted line) in hexane (25). All action spectra were corrected for quantum energy and for absorption by the solution and by the front cuvette wall. Also shown are the absorption spectra (light solid lines) of NADH in tris(hydroxymethyl)aminomethane buffer (pH 9.5) and ethanol (24) and of vitamin K_2 in isooctane (10). All spectra are normalized to a maximum value of 1.0.*

TABLE 2. *Inhibition of various E. coli systems by 334-nm radiation*

System	Strain	F_{37} (kJ/m²)	Reference
Growth	B	19, 25[a]	(13, 18)
	AB2463	23[a]	(17)
NADH dehydrogenase	B	40	This paper
Net ATP synthesis (in succinate)	AB2463	140	This paper
Ubiquinone Q-8 in hexane		100	(25)
Ubiquinone Q-8 in vivo	B	420	(25)

[a] These values were calculated by the "extrapolation method" described by Jagger (11).

coli. Jones (14), for example, showed that ubiquinone Q-8 rather than vitamin K was involved in the oxidation of NADH in *E. coli* strain 156:53D2.

Bragg and Hou (4) found that near-UV irradiation of a "small particle" fraction of *E. coli* results in rapid loss of NADH oxidase activity (measured as loss of 340-nm absorption by added NADH), and the activity cannot be restored by addition of flavins or quinones. However, this "small particle" fraction appears to represent only a fragment of the normal electron transport system, since it lacks cytochromes *a* and a_2 and vitamin K and has little ubiquinone Q-8 and no succinate, formate, pyruvate, or NADH phosphate oxidase activities. Subsequent work with a more complete fraction (5) showed that NADH-cytochrome b_1 reductase activity was inactivated by near UV (although the NADH-flavin reductase activity was not). Addition of flavins did not restore activity to the cytochrome b_1 system, but addition of menadione resulted in a considerable increase. Still later work (3) showed that the NADH oxidase system suffers near-UV lesions both between NADH and cytochrome b_1 and between cytochrome b_1 and oxygen. The labile site in the latter segment appeared to be cytochrome a_2, but the site in the former segment was not identified. They proposed that ubiquinone is present in both segments and is only partly inactivated at fluences that destroy the cytochrome a_2. It is to be noted that the source used by Bragg and co-workers emits much radiation around 410 nm, which is well absorbed by cytochromes. This contrasts with our action spectra, which show no evidence of cytochrome-like absorption, which is low at 340 nm and high at 430 nm.

Cox et al. (8) interpret their own results in a rather similar way. They suggest that ubiquinone occurs both before and after cytochrome b_1 in their *E. coli* K-12 strains, and that the ubiquinone is not a direct electron carrier but is complexed with some other electron carrier, presumably nonheme iron.

We conclude from these reports that neither the location nor the roles of the quinones have yet been clearly established in *E. coli*.

ATP synthesis. Impaired oxidative phosphorylation (ATP synthesis) could result from damage to intermediates in the oxidative pathway, resulting in a decreased level of ATP in the cell. The data of Fig. 5 show that the action spectrum for inhibition of net ATP synthesis in succinate in *E. coli* AB2463 is somewhat similar to the action spectra reported by Werbin et al. (25) for inactivation of ubiquinone Q-8 either in vivo or in vitro. Also, the fluence required for inhibition of ATP synthesis is intermediate between those required for inactivation of ubiquinone Q-8 in vivo and in vitro (Table 2). The inhibition of ATP synthesis observed in the present experiments may therefore be due to absorption of light in ubiquinone Q-8 and subsequent destruction of the quinone. That is, ubiquinone could be both a chromophore and a target molecule.

Vitamin K is also a possible chromophore. Brodie (6) found vitamin K_1 to be much more sensitive to 360-nm light than ubiquinone Q-10. Vitamin K_2 also appears to be more sensitive (10), and its absorption spectrum is quite similar to the action spectrum for inhibition of ATP synthesis (Fig. 5).

Finally, that inhibition of net ATP synthesis in succinate might be accounted for on the basis that either or both ubiquinone Q-8 and vitamin K_2 are chromophores would not be inconsistent with the rough similarity of the absorption spectrum for vitamin K_2 and the action spectra for ubiquinone Q-8 destruction, and with the experimental error inherent in our determination of the action spectrum for inhibition of ATP synthesis.

It is remarkable that NADH dehydrogenase activity is so much more sensitive to near UV than is ATP synthesis (Table 2). However, we measured ATP synthesis in a succinate medium, where the cells presumably would not use the NADH dehydrogenase system. Therefore, our results are still consistent with ubiquinone Q-8 being a chromophore for inhibition of ATP synthesis in succinate. One then might wonder if ATP synthesis in a medium that utilized the NADH pathway would be much more sensitive than in succinate. Actually, the opposite would appear to be true, since ATP synthesis in pyruvate (Table 1) is much more resistant than in succinate. We do not understand this result; one would expect the NADH pathway to be inactivated earlier than the succinate pathway, since vitamin K is more sensitive than ubiquinone, and pyruvate-dependent ATP synthesis ought to show at least the sensitivity of succinate-dependent synthesis.

The absorption spectrum of NADH resembles neither the action spectrum for inhibition of NADH dehydrogenase nor the action spectrum for inhibition of ATP synthesis, and we therefore conclude that NADH itself is not serving as a chromophore that absorbs energy and passes it on to another (target) molecule which is inactivated. That NADH might itself be damaged is unlikely, since it shows a very high resistance to near-UV inactivation in vitro (unpublished data).

The fact that ATP synthesis is resistant to near UV in the presence of glucose (Table 1) is not surprising, since Hempfling (9) has shown that oxidative phosphorylation in *E. coli* is repressed by glucose. We therefore assume that ATP synthesis in the presence of glucose is occurring by fermentation, although we have not checked this point.

NADH dehydrogenase. The NADH dehydrogenase activity of *E. coli* extracts is quite sensitive to near-UV radiation (Table 2). To confirm that near-UV inactivation of NADH dehydrogenase activity also occurs in whole cells, we irradiated intact cells of *E. coli* and found that inactivation also occurred, with roughly the same efficiency as in vitro.

The action spectrum for loss of NADH dehydrogenase activity of crude cell extracts (Fig. 5) is quite different from the absorption spectrum of vitamin K_2, but it does resemble the action spectrum for photoalteration of Q-8 in vivo. However, Q-8 destruction, both in hexane and in cells, is much more resistant (Table 2).

We conclude that, although ubiquinone Q-8 could (from the action spectrum data) be the chromophore for loss of NADH dehydrogenase activity, it clearly is not a target molecule, since the fluences required for destruction of Q-8 and of NADH dehydrogenase are so different (Table 2). The action spectrum also rules out NADH itself as either a chromophore or a target, and its insensitivity to near UV in vitro further supports this idea.

Growth delay. We have earlier suggested (10) that the oxidative phosphorylation system might contain the target for near-UV-induced growth delay in *E. coli*. However, we now find that the doses required for inhibition of growth are only one-sixth of those required for inhibition of ATP synthesis (Table 2). In addition, there is now strong evidence that growth delay is induced by action on transfer ribonucleic acid (21; Ramabhadran and Jagger, Proc. Natl. Acad. Sci. U.S.A., in press). Finally, the action spectrum for inhibition of ATP synthesis (Fig. 5) is different from that for inhibition of growth, which shows a peak of efficiency at 334 nm and no effect at all at 405 nm (13). We therefore conclude that the oxidative phosphorylation system is not a primary factor in the induction of growth delay in *E. coli* by near UV.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grant AM14893 from the National Institute of Arthritis, Metabolism, and Digestive Diseases and by The University of Texas at Dallas Research Fund.

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