JOURNAL OF BACTERIOLOGY, Sept. 1974, p. 899-906

Vol. 119, No. 3
Printed in U.S.A.

Photoinactivation of Ammonia Oxidation in *Nitrosomonas*

ALAN B. HOOPER AND KATHLEEN R. TERRY

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

Received for publication 15 April 1974

Photoinactivation of ammonia oxidation in cells of *Nitrosomonas* was shown to follow first-order kinetics with a rate constant proportional to incident light intensity. The action spectrum for photoinactivation consisted of a broad peak in the ultraviolet range, where both hydroxylamine and ammonia oxidation were affected, and a shoulder at approximately 410 nm where only ammonia oxidation was affected. In photoinactivated cells, hydroxylamine but not ammonia was oxidized to nitrite and hydroxylamine but not ammonia caused reduction of cytochromes in vivo. The amount per cell of the following constituents was not measurably altered by photoinactivation: cytochromes *b*, *c*, *a*, and P460; ubiquinone; phospholipid; free amino acids; hydroxylamine-dependent nitrite synthetase; nitrite reductase; *p*-phenylenediamine oxidase; and cytochrome *c* oxidase. Malonaldehyde or lipid peroxides were not detected in photoinactivated cells. Photoinactivation was prevented (i) under anaerobic conditions, (ii) in the presence of methanol, allylthiourea, thiosemicarbazide, hydroxylamine, ethylxanthate, or CO at concentrations wich caused 100% inhibition of ammonia oxidation, and (iii) at concentrations of ammonia or hydroxylamine which gave a rapid rate of nitrite production. Recovery of ammonia oxidation activity in 90% inactivated cells took place in 6 h, required an energy and/or nitrogen source, and was inhibited by 400 μg of chloramphenicol per ml.

The difficulty in obtaining cell-free extracts capable of oxidizing ammonia to nitrite at high rates has resulted in a dependence on observations with intact cells for knowledge of the mechanism of ammonia oxidation in *Nitrosomonas*. Studies of the kinetics (11) and the effect of inhibitors (13) on ammonia oxidation by *Nitrosomonas* have indicated that the reaction probably involves (i) a preincubation-inducible state, (ii) a metal ion such as copper, (iii) a CO-binding factor, (iv) a functionally intact membrane, and (v) a free radical or a free radical-sensitive enzyme or chemical intermediate. Ammonia oxidation but not hydroxylamine oxidation is inactivated by illumination with visible light (13). In the present work, the characteristics of photoinactivation have been determined with the aim of obtaining information bearing on the nature of the ammonia oxidation system. The results suggest that photoinactivation involves the absorption of light at 410 nm and subsequent photooxidative damage to an enzyme specific to ammonia oxidation, that the enzyme is in a relatively sensitive state during endogenous nitrite production or slow ammonia oxidation, and that a reactive N- or O-containing chemical intermediate of ammonia oxidation may be involved.

MATERIALS AND METHODS

Growth of cells and preparation of extracts. Cultures of *Nitrosomonas europaea* (Schmidt strain) were grown in liquid medium containing: Na_2HPO_4, 93.6; KH_2PO_4, 6; $NaHCO_3$, 6; and $(NH_4)_2SO_4$, 20 mM. Trace elements, added separately at time of inoculation, consisted of: $MgCl_2$, 2.03; $CaCl_2$, 0.13 mM; and ferric chelate (Sequestrene-138, Geigy Agricultural, Yonkers, N.Y.), 0.01 mM. Cell-free extracts were prepared as described previously (12).

Extraction and identification of lipids. Cells (100 mg wet weight) were extracted with 20 ml of chloroform-methanol (2:1, vol/vol) and shaken overnight at 4 C on a wrist-action shaker. Phases were separated by centrifugation, and the aqueous phase was reextracted for 15 min with chloroform-methanol. The combined organic phases were evaporated to dryness under a stream of nitrogen and dissolved in 0.5 ml of methanol-benzene (1:1, vol/vol). Samples were spotted on 0.4-mm silica gel H plates and developed in petroleum ether, diethylether, acetic acid (85:15:1, vol/vol/vol) to separate lipid groups. Plates were sprayed with sulfuric acid and charred at 170 C. For gas-liquid chromatography, methyl esters were prepared from the methanol-benzene residue (8), and samples were applied to a F & M model 5750 hydrogen flame chromatograph (4).

Extraction and analysis of amino acids. Cells (100 mg wet weight) were suspended in 2 ml of 80% ethanol, disrupted with a Branson sonifier, and

extracted at 60 C for 1 h. After centrifugation, the residue was reextracted with 1 ml of ethanol for 15 min. The extracts were pooled and reduced to a volume of 1 ml under a stream of nitrogen. Total ninhydrin-positive material was estimated according to the method of Clark (5). For qualitative estimation of individual amino acids, 100 μliters of extract was spotted on Whatman no. 1 filter paper and developed by descending chromatography in a solvent system consisting of butanol, acetic acid, and water (65:25:10, vol/vol/vol). After drying, paper was sprayed with 0.1% ninhydrin in acetone, and the spots were developed at 50 C.

Assay procedures. The rate of nitrite production from ammonia or hydroxylamine by cells was determined as described previously (13) in a reaction mixture containing 0.2 mg (wet weight) of *Nitrosomonas* cells per ml and 5×10^{-4} M $(NH_4)_2SO_4$, or 10^{-3} M NH_2OH in 0.05 M phosphate, pH 7.5. Samples were removed at regular intervals and assayed for nitrite by the diazotization method (15). Enzyme assays have been described previously: hydroxylamine-dependent nitrite synthetase (13), cytochrome oxidase (7), nitrite reductase (10), *p*-phenylenediamine oxidase (12), and ubiquinone (12). Absorption spectra were determined in an Aminco DW-2 scanning spectrophotometer. Spectra were determined at the temperature of liquid nitrogen (77 K) with cells or extracts suspended in 50% glycerol. Malonaldehyde was estimated by the thiobarbiturate method (16).

Illumination of cells. Unless otherwise indicated, cells were suspended (0.4 mg wet weight/ml) for illumination in 0.05 M phosphate solution, pH 7.5, in a 12-mm diameter test tube or 1.0-cm path-length quartz or glass cuvette. The temperature was maintained at 25 C. Samples of the cell suspension were removed at regular intervals and added to an equal volume of phosphate solution containing 1 mM ammonium sulfate or 2 mM hydroxylamine, and the rate of nitrite production was determined in the dark. The time ($t_{½}$ min) for cells to lose half their nitrite-producing ability was determined from a semilogarithmic plot of rate of nitrite production as a function of time. The value of the first-order rate constant for photoinactivation was $K = 0.693/t_{½}$ min^{-1}. Incident light intensity was measured under identical conditions as the photoinactivation as the rate of photoreduction of iron in 0.15 M potassium ferrioxalate solution (9). Unfiltered light was from a Kodak model 850 Carousel projector with a 500-W General Electric DEK lamp. In the visible range, monochromatic light was selected with a Bausch and Lomb 500-mm model grating monochromator. In the ultraviolet range, monochromatic light was selected with a Farrand Foci-Flex grating monochromator with a 150-W Xenon arc light source.

RESULTS

Kinetics, dose response, and action spectrum. As shown in Fig. 1, the time course of photoinactivation was first order, with respect to the time of illumination, with unfiltered light (Fig. 1A) as well as with light of a narrow wavelength range at all cases where inactivation was observed above 250 nm. The time course of photoinactivation at 410 nm is shown in Fig. 1B.

The first-order rate constant for photoinactivation was proportional to the incident light intensity (Fig. 2). This relationship held for unfiltered light or light of selected wavelengths over the range studied above 250 nm. As an example, the dose response for photoinactivation at 410 nm is shown by the open circles in Fig. 2. The total dose of light required to effect 50% inactivation of ammonia oxidation was approximately 2.8×10^{-7} Einsteins/cm^2 or 5.5×10^{-6} Einsteins/cm^2 for monochromatic (410 nm) or unfiltered light, respectively.

Figure 3 shows the action spectrum for photoinactivation of hydroxylamine- or ammonia-oxidizing ability in *Nitrosomonas*. First-order rate constants for photoinactivation were measured at selected wavelengths and normalized with respect to the incident light intensity determined by actinometer measurement under the same conditions. At wavelengths below 400 nm both ammonia and hydroxylamine oxidation activity were destroyed by illumination. In the wavelength range of 400 nm and above, photoinactivation of hydroxylamine-oxidizing ability did not occur; in fact, a 10% stimulation was generally observed. In contrast, ammonia oxidation was strongly inactivated in the wavelength range 400 to 410 nm. Light at wavelengths greater than 430 nm caused little or no inactivation.

The nitrite-oxidizing ability of a suspension of cells of *Nitrobacter agilis* (Schmidt) was not impaired after illumination under conditions where ammonia oxidation in *Nitrosomonas* was 100% inactivated.

Changes in cellular constituents. Although peaks representative of the reduced forms of the major *b*-, *c*-, and *a*-type cytochromes were observed in non-illuminated cells with ammonia or hydroxylamine as reductant and illuminated cells with hydroxylamine as reductant, they were not observed in illuminated cells in the presence of ammonia.

Dithionite-reduced-*minus*-oxidized difference spectra of control or illuminated cells at 77 K or at 25 C were essentially identical with respect to the content of the major peaks representative of the *b*-, *c*-, *a*- and P-460 type cytochromes, indicating that there had not been photo-damage of a detectable fraction of any type of cytochrome. Likewise, reduced-plus-CO-*minus*-reduced (25 C) difference spectra of

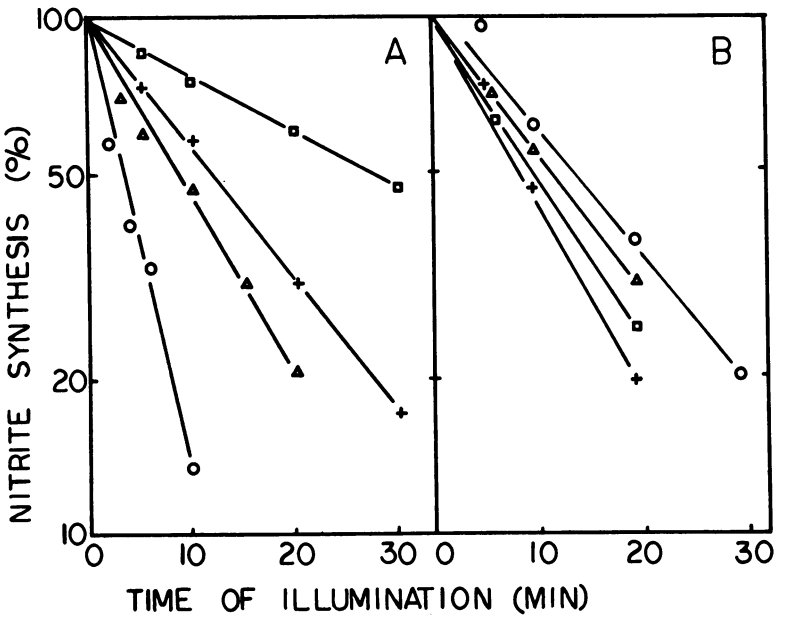

FIG. 1. *Time course of photoinactivation of ammonia oxidation. Cells were illuminated and the rate of nitrite production was assayed as indicated in Materials and Methods. (A) Unfiltered light. Incident light intensity was varied by adjusting the distance between light source and cells. Symbols: 10* (○); *20* (△); *25* (+); *and 32* (□), *inches from light source, respectively. (B) Monochromatic light, wavelength 410 nm. Incident light intensity was varied by adjusting the width of the exit slit. Symbols: 13-mm slit* (+), *10-mm slit* (□), *7.5-mm slit* (△), *and 5-mm slit* (○).

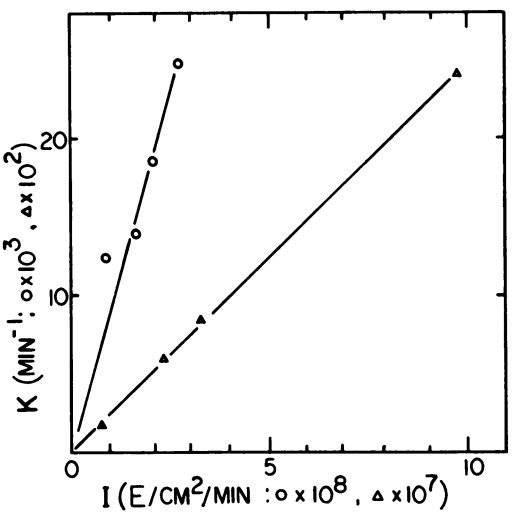

FIG. 2. *Rate of photoinactivation of ammonia oxidation as a function of incident light intensity. The first-order rate constant for each point was calculated from a time course of inactivation measured as in Fig. 1. The incident light intensity was measured by the ferrioxalate actinometer as described in Materials and Methods. Symbols:* (△), *unfiltered light, intensity varied by the distance from the light source;* (○), *monochromatic light, 410 nm, intensity varied by the exit slit width of the monochromator.*

photoinactivated and control cells were very similar with respect to the absorbancy maximum at 414 nm and troughs at approximately 548 and 556 nm, indicative of the content of an *o*-type cytochrome, and the maximum at 450 nm and minimum at 463 nm indicative of P-460 of *Nitrosomonas* (6). The activities per milligram of protein of the enzymes, hydroxylamine-dependent nitrite synthetase, nitrite reductase, cytochrome oxidase, and *p*-phenylenediamine oxidase, were identical in extracts of illuminated cells and cells which had been completely inactivated with unfiltered visible light.

That the permeability of photoinactivated cells was not drastically changed was indicated by the equal contents of ethanol-soluble ninhydrin-positive material in control and 100% photoinactivated cells. Membranes of *Nitrosomonas* were found to contain 16:0 and 16:1 fatty acids as the most prevalent species with a small amount of 18:0 fatty acid, in keeping with previous observations (1). By thin-layered chromatography, essentially all fatty acid was in the form of phospholipid. The content of phospholipid, 16:0, 16:1, and 18:0 fatty acid and ubiquinone in control, and 100% photoinactivated cells was essentially the same. The gas-liquid chromatography elution profile of

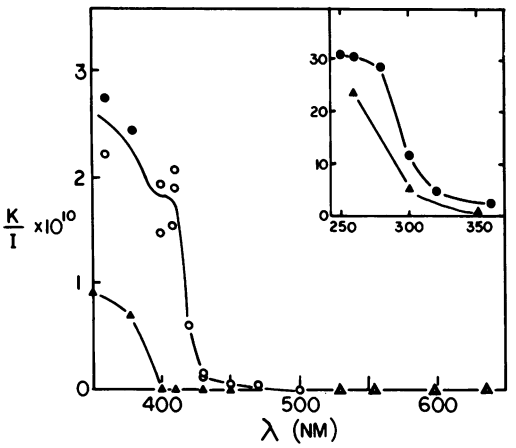

FIG. 3. *Action spectrum for photoinactivation of ammonia oxidation. Washed Nitrosomonas cells were illuminated in a 3-ml quartz cuvette. The rate constant, K min⁻¹, for photoinactivation was measured and the incident light intensity, I (Einsteins per cm² per min), was calculated under the same conditions at each wavelength as described in Materials and Methods. Inactivation of nitrite synthesis, ammonia as substrate; Farrand Foci-Flex grating monochromator, 150-W Xenon arc lamp (●), Bausch and Lomb grating monochromator (○). Inactivation of nitrite synthesis, hydroxylamine as substrate; Farrand (▲), Bausch and Lomb (△).*

fatty acids did not show detectable peaks of the hydroperoxides or scission products of 16- or 18-carbon fatty acids, nor was malonaldehyde found in extracts of 100%-inactivated cells.

Variation in sensitivity to photoinactivation. For an unknown reason, the amount of illumination to effect 50% inactivation varied by 10 to 15% between batches of cells. Growth of cells into stationary phase of growth in the presence of increased amounts of chelated iron resulted in cells which were less sensitive to photoinactivation, whereas growth in the absence of added iron yielded cells which were nearly twice as sensitive as cells grown by the usual procedure (Table 1). The altered sensitivity was not due to a major change in cytochrome content as indicated by the similarity of absorbancy at 420, 460, 530, and 550 nm in spectra of dithionite-reduced extracts of cells grown in varied levels of iron. The growth rate, cell yield, and rate of nitrite production were likewise unaffected by varying the iron content during the two transfers. Presumably, iron carried in with the first inoculation was sufficient to give normal growth.

Protection from photoinactivation. Under the usual conditions of photoinactivation, ammonia or hydroxylamine were not added. Under those conditions, cells produced nitrite at approximately 0.5% of the rate in the presence of added substrate. The nitrogen source for this endogenous nitrite production is unknown. The presence of 20 mM ammonia or 1 mM hydroxylamine during illumination decreased the extent of photoinactivation (Table 2). The conditions which afforded greatest protection were those in which nitrite was produced at the greatest rate during illumination. When oxygen was absent or in very low concentration during illumination, cells were completely protected from photoinactivation. These results suggest that inactivation involved a light-induced reaction with oxygen, although protection may also have been an indirect effect of the absence of nitrite production during illumination.

Cells were protected from photoinactivation in the presence of one of a number of inhibitors of the oxidation of ammonia to nitrite (Table 3). This was true at concentrations of inhibitors which completely inhibited nitrite production from 1 mM ammonia in the standard assay or during illumination. At concentrations of inhibitors where inhibition of ammonia oxidation in the standard assay was not complete, photoinactivation was potentiated (Table 4). An exception to this potentiation was methanol, which protected the cells from photoinactivation to some degree at concentrations which gave incomplete levels of inhibition of ammonia oxidation.

Recovery of cells from photoinactivation. Photoinactivated cells in 0.05 M phosphate solution, pH 7.5, at 4 or 25 C did not increase in

TABLE 1. *Effect of chelated iron content of growth medium on sensitivity to photoinactivation*[a]

Added Fe[b] (mM)	$t_{\frac{1}{2}}$ (min)
0	16.0
0.001	23.5
0.01[c]	27.0
0.1	29.0
1.0	31.2

[a] Cells were cultured by inoculating 1 ml of a rapidly growing culture into 100 ml of medium containing variable levels of iron chelate. After 5 days, these subcultures were transferred to 1 liter of the same medium. After 6 days of subsequent growth, when essentially all the ammonia in the medium had been oxidized to nitrite, cells were harvested, and sensitivity to light was determined by illumination utilizing the Bausch and Lomb monochromator at 410 nm, as previously described.

[b] As Sequestrene 138-Fe which contains 6% Fe.

[c] Concentration normally present in the growth medium.

TABLE 2. *Protection from photoinactivation in the presence of substrate or absence of oxygen*[a]

Condition during illumination	NO_2^- per min during 30-min illumination		NO_2^- per min after illumination			
			NH_3 as substrate[b]		NH_2OH as substrate[c]	
	nmol/min	% Control[d]	nmol/min	%	nmol/min	%
No added substrate	0.01	0.4	0.02	1	29	106
Ammonia (1 mM)	3	12	0.6	2		
Ammonia (20 mM)	18	45	14.6	36		
Hydroxylamine (1 mM)[e]	30.8	110	16.9	66	30.8	110
Anaerobiosis	0	0	26.4	103		

[a] Cells were illuminated with unfiltered light as described in Materials and Methods under the conditions indicated in the table. After illumination the indicated substrate was added, and rate of nitrite synthesis was measured.

[b] 1 mM NH_3 added after illumination.

[c] 1 mM NH_2OH added after illumination.

[d] Control cells (not illuminated) produced HNO_2 at the following rates (in nmol per min per ml): 1 mM NH_3, 25.5; 20 mM NH_3, 40; 1 mM NH_2OH, 28.

[e] These cells were washed free of NH_2OH before nitrite production from ammonia was assayed. Nitrite produced during illumination was derived from hydroxylamine present.

TABLE 3. *Protection from photoinactivation in the presence of inhibitors*[a]

Inhibitor	Concn of inhibitor (mM)	Activity $NH_3 \rightarrow HNO_2$	
		Non-illuminated cells (%)[b]	Illuminated cells after washing (%)[c]
None	0	100	45
Allylthiourea	1.0	0	100
Thiosemicarbazide	1.0	2	86
Carbon monoxide	50	0	125
Sodium sulfide	1.0	0	92
Methanol	250	0	116
Sodium dithionite	10	0	103
Dichlorophenol-indophenol	1.0	0	86

[a] Cells (5 mg/ml wet weight) were suspended in 4 ml of 0.05 M phosphate, pH 7.5, in the presence of the indicated concentration of inhibitor.

[b] "Non-illuminated" control cells were maintained in the dark for 30 min. The rate of nitrite production was assayed and compared with the rate in the absence of inhibitor.

[c] "Illuminated" cells were illuminated for 30 min with the Kodak projector and washed 3 times by sedimentation at 10,000 × *g* for 10 min and resuspended in 5 ml of phosphate solution. Washed cells were suspended in 5 ml of phosphate solution, 1 mM ammonia, and the rate of nitrite production was compared with the rate catalyzed by cells which had been incubated without illumination for 30 min in the absence of inhibitor, washed, and resuspended.

TABLE 4. *Potentiation of photoinactivation*

Inhibitor	Concn (M)	Activity $NH_3 \rightarrow HNO_2$[a]	
		Non-illuminated cells (%)	Illuminated cells after washing (%)
None	0	100	45
Allylthiourea	5×10^{-6}	7	23
	1×10^{-5}	1	34
	5×10^{-4}	0	90
	1×10^{-3}	0	100
Sodium dithionite	1×10^{-3}	[b]	6
	1×10^{-2}	0	103
Methanol	5×10^{-3}	27	56
	1.3×10^{-2}	7	64
	5×10^{-2}	1	88
	2.5×10^{-1}	0	116
Cold	4 C	1[c]	30

[a] Percent activity of non-illuminated and illuminated cells determined as in Table 3.

[b] Dithionite inhibition decreased with time. Initial rate at this concentration was inhibited by 95%.

[c] As compared with rate at 25 C.

ability to oxidize ammonia during incubation for as long as 24 h. As shown in Fig. 4, when inoculated into growth medium, 90%-inactivated cells recovered the ability to oxidize ammonia to nitrite after a delay of 6 h with full activity present after 10 h. That the increase in rate of nitrite production was due to recovery of existing cells rather than the selective growth of non-inactivated cells was indicated by the fact that (i) the rate of nitrite production after recovery greatly exceeded the rate of nitrite

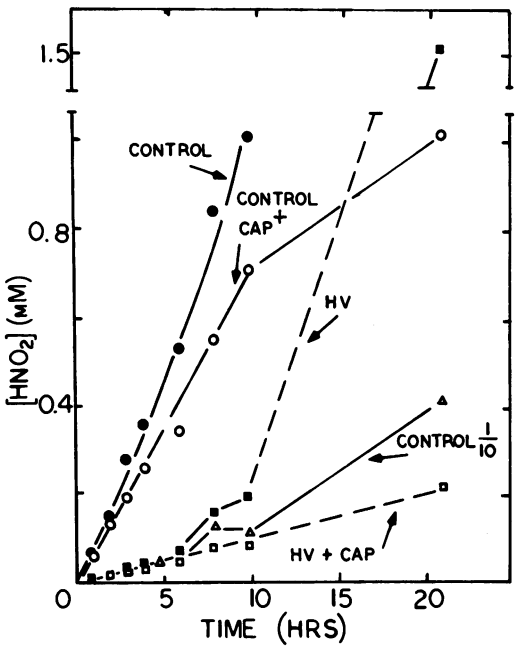

FIG. 4. *Recovery of illuminated cells and effect of chloramphenicol on recovery. Harvested cells were suspended in phosphate buffer at a concentration of 2 mg (wet weight) per ml. The suspension was either stored in the dark or illuminated for 45 min at an intensity approximately 3 × 10^{-7} Einsteins per cm^2 per min, with a Kodak model 850 Carousel slide projector with a 500-W tungsten lamp. After illumination, cells produced nitrite from 0.5 mM ammonium sulfate at one-tenth the rate of the control cells stored in the dark. After treatment, cells were inoculated into 50 ml of growth medium at a final concentration of 40 μg (wet weight) per ml. Nitrite production was followed from the time of inoculation to 22 h. Symbols: unilluminated control cells (●); control cells, plus 400 μg of chloramphenicol per ml in growth medium (○); illuminated cells (■); illuminated cells plus 400 μg of chloramphenicol per ml in growth medium (□); control cells, inoculated at a final concentration of 4 μg (wet weight) per ml (△).*

production in an unilluminated culture which had been diluted by 1/10 so as to produce nitrite at the same rate as the photoinactivated culture, and (ii) cells which were 100% photoinactivated (produced no nitrite from 1 mM ammonia during a 1-h incubation after 12 or 24 h of preincubation in phosphate solution) fully recovered the ability to produce nitrite after 10 h of incubation in growth medium. Although not shown in Fig. 4, cells recovered completely in phosphate solution in the presence of low (0.2 mM) ammonia. After recovery, cells grew at the same rate and to the same final level of nitrite and protein in the culture as unilluminated cells. The ability of photoinactivated cells to regain ammonia-oxidizing activity was completely prevented in the presence of 400 μg of chloramphenicol per ml (Fig. 4), indicating that protein synthesis was necessary for recovery and thus that a protein may have been damaged during photoinactivation.

DISCUSSION

The data do not establish the site of damage or the nature of the photoreaction or allow an explanation of the variation of sensitivity with cell metabolism during illumination. We present the working model shown in Fig. 5 to summarize and rationalize the observations in at least a formal sense.

Nature of damage. The damage resulting from illumination with light at wavelengths between 400 and 430 nm was specific to the ammonia oxidation system and did not include major disruption of membrane permeability or destruction of lipids, ubiquinone, *b*-, *c*-, *a*-, *o*-, or P-460-type cytochromes, hydroxylamine-dependent nitrite synthetase, or the membrane-bound cytochrome oxidase. Damage to a small amount of one of these components which was critically associated with the ammonia oxidase system cannot be ruled out. Recovery of ammonia oxidation ability was inhibited by chloramphenicol, which suggests that a damaged factor was a protein. The apparent requirement for oxygen of photoinactivation suggests that light-induced oxidation of a critical protein was involved. By analogy with other photooxidative reactions in biology, it is reasonable to postulate that a peroxide or free radical is involved and that the sensitive protein is located in a relatively nonaqueous environment such as the membrane.

Nature of inactivating reaction. The sensitivity of both ammonia and hydroxylamine oxidation to illumination in the ultraviolet

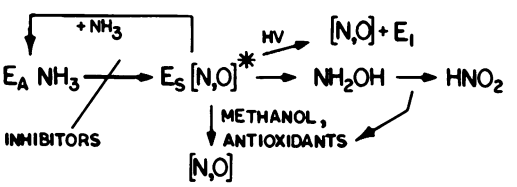

FIG. 5. *Working model for photoinactivation of ammonia oxidation in Nitrosomonas. E_a, E_s, and E_i: active, sensitive, and inactive enzyme, respectively. [N, O]* and [N, O]: reactive and nonreactive N- and/or O-intermediates or biproducts of ammonia oxidation, respectively. Inhibitors of ammonia oxidation include allylthiourea, thiosemicarbazide, hydroxylamine, ethylxanthate, and carbon monoxide.*

range may have involved general damage resulting from lipid or ubiquinone peroxidation, as has been observed in the photoinactivation of other electron transport systems (2), and may be associated with the high content of *Nitrosomonas* of such electron transport components as ubiquinone, membrane (and thus, presumably, lipid), and cytochromes (12). Because specific damage to the ammonia oxidation system was induced by light at wavelengths between 400 and 430 nm, the light-absorbing factor is probably a porphyrin, heme, or hemoprotein, possibly the light-sensitive protein itself. Porphyrins have been implicated in the photoinactivation of growth of slime molds (3) and cholesterol hydroperoxide formation in red cell membranes (14). A plausible hypothesis to explain the decreased photosensitivity resulting from growth of cells in increasing concentrations of iron is that the steady-state level of porphyrins decreased as heme was available in high enough concentrations to inhibit or repress enzymes of porphyrin synthesis.

Basis for variation in sensitivity. Cells were less sensitive to photoinactivation in the absence of oxygen and in the presence of inhibitors at concentrations which resulted in 100% inhibition of nitrite production. These are conditions in which the flux through the ammonia-to-hydroxylamine-to-nitrite pathway was zero. Cells were likewise less sensitive to photoinactivation in the presence of high concentrations of ammonia or of hydroxylamine, conditions of great flux through ammonia and/or hydroxylamine oxidative pathways. Cells were especially sensitive to photoinactivation during endogenous nitrite production, at low ammonia concentrations, at decreased temperatures, and in the presence of inhibitors at concentrations giving less than 100% inhibition of ammonia oxidation. These conditions allowed a slow flux through the ammonia-to-hydroxylamine-to-nitrite pathway. The effect of protective conditions such as the absence of O_2 or the presence of allylthiourea may also be a direct result of anaerobiasis or a protective reaction involving allylthiourea.

To explain why photoinactivation appeared to require a slow flux through the ammonia oxidation pathway, we suggest that the target enzyme participated in a light-activated oxidative reaction with reactive intermediates of the ammonia oxidation pathway ([N, O]*, Fig. 5) which were in close proximity to the enzyme. Such intermediates may have been N- and/or O-containing free radicals which were also reactive with added trapping agents, such as dithionite or methanol (which protected from photoinactivation while allowing nitrite synthesis), or with an endogenous protective compound (free radical trapping agent or antioxidant) produced during hydroxylamine oxidation. According to this model, under conditions of complete absence of ammonia oxidation due to the presence of inhibitors or the absence of oxygen, the photoreactive intermediates [N, O]* were not present. In the presence of high concentrations of ammonia and high flux through the hydroxylamine-to-nitrite pathway, enough of an excess of antioxidant may have been generated to trap photoreactive compounds or reactive compounds generated under illumination. Other possibilities consistent with the present observations are (i) that the enzyme was in a transient sensitive (possibly oxidized) state (E_s) during nitrite synthesis, but was shifted to a more stable (possibly reduced) form in the presence of dithionite or high flux through the hydroxylamine oxidation pathway and/or (ii) that ammonia displaced reactive intermediates from the enzyme and thereby returned the enzyme to a non-photosensitive state (E_a).

ACKNOWLEDGMENTS

We thank Peter Maxwell for enzyme assays, E. L. Schmidt for *Nitrobacter agilis* cultures, and Rex Lovrien, Robert Glass, and Albert Frenkel for helpful and stimulating discussion.

This investigation was supported by research grants from the National Science Foundation (GB40411) and from the Graduate School, University of Minnesota.

LITERATURE CITED

1. Blumer, M., T. Chase, and S. W. Watson. 1969. Fatty acids in the lipids of marine and terrestrial nitrifying bacteria. J. Bacteriol. **99**:366-370.
2. Bragg, P. D. 1971. Effect of near-ultraviolet light on the respiratory chain of *Escherichia coli*. Can. J. Biochem. **49**:492-495.
3. Burchard, R. P., and M. Dworkin. 1966. Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. J. Bacteriol. **91**:535-545.
4. Christopherson, W. S., and R. L. Glass. 1969. Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. J. Dairy Sci. **52**:1289-1290.
5. Clark, J. M. 1964. Experimental biochemistry, p. 95. W. H. Freeman, San Francisco.
6. Erickson, R. H., and A. B. Hooper. 1972. Preliminary characterization of a variant CO-binding heme protein from *Nitrosomonas*. Biochim. Biophys. Acta **275**:231-244.
7. Erickson, R. H., A. B. Hooper, and K. R. Terry. 1972. Solubilization and purification of cytochrome a_1 from *Nitrosomonas europaea*. Biochim. Biophys. Acta **283**:155-166.
8. Glass, R. L. 1971. Alcoholysis, saponification and the preparation of fatty acid methyl esters. Lipid **6**:919-925.
9. Hatchard, C. G., and C. A. Parker. 1936. A new scientific chemical actinometer. II: Potassium ferrioxalate as a standard chemical actinometer. Proc. Roy. Soc. Ser. A **235**:518-536.

10. Hooper, A. B. 1968. A nitrite-reducing enzyme from *Nitrosomonas europaea*. Biochim. Biophys. Acta **162**:49–65.
11. Hooper, A. B. 1969. A lag phase of ammonia oxidation by resting cells of *Nitrosomonas europaea*. J. Bacteriol. **97**:968–969.
12. Hooper, A. B., R. H. Erickson, and K. R. Terry. 1972. Electron transport systems of *Nitrosomonas*: isolation of a membrane-envelope fraction. J. Bacteriol. **110**:430–438.
13. Hooper, A. B., and K. R. Terry. 1973. Specific inhibitors of ammonia oxidation in *Nitrosomonas*. J. Bacteriol. **115**:480–485.
14. Lamola, A. A., T. Yamane, and A. M. Trozzolo. 1973. Cholesterol hydroperoxide formation in red cell membranes and photohemolysis in erythropoietic protoporphyria. Science **179**:1131–1133.
15. Nicholas, D. J. P., and A. Nason. 1957. Determination of nitrate and nitrite, p. 981–984. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
16. Ottolenghi, A. 1959. Interaction of ascorbic acid and mitochondrial lipids. Arch. Biochem. Biophys. **79**:355–363.