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Spectroscopic evidence for a photosensitive oxygenated state of ammonia mono-oxygenase

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Photoinactivation of ammonia oxidation by *Nitrosomonas europaea* cells by near-u.v. light was confirmed and further shown to occur with the same rate constant as loss of bromoethane-oxidation activity. Hydroxylamine oxidation was much less photosensitive. Protection against inactivation was afforded by anaerobiosis, organic substrates of ammonia mono-oxygenase such as bromoethane, or metal-ion-chelating agents such as thiourea. The presence of 10mM-NH_4^+ or 1mM-hydroxylamine made little difference, whereas hydrazine had a potentiating effect. Illumination of cells also caused a bleaching in the absorption spectrum around 380nm, along with changes in the cytochrome γ-band region. Similar effects below 400nm were obtained when organic substrates and inhibitors of the mono-oxygenase were added to cells in the dark. The copper proteins haemocyanin and tyrosinase have a photosensitive oxygenated state with a near-u.v. absorption band of similar half-width. They also have a sensitivity to chelating agents similar to that of ammonia mono-oxygenase. The experimental results are explained in terms of a three-stage catalytic cycle analogous to that for tyrosinase. In resting cells most of the enzyme is believed to be in an oxygenated (Oxy) form, which absorbs maximally at 378nm and is photosensitive. In the presence of a substrate, one O atom is inserted into the substrate and the other is reduced to water, leaving the enzyme in an oxidized (Met) state. This is followed by a two-electron reduction of the proposed binuclear copper site to give a reduced (Deoxy) state, which can bind O_2 to complete the cycle.

The biological conversion of NH_4^+ ions to nitrite is mainly brought about by a specialized group of chemolithotrophic bacteria, typified by *Nitrosomonas europaea*. The ammonia is first converted into hydroxylamine by ammonia mono-oxygenase (see Hyman & Wood, 1983):

$$NH_3 + 2[H] + O_2 \rightarrow NH_2OH + H_2O$$

The further oxidation of hydroxylamine provides energy for the cells, and must in addition furnish the mono-oxygenase with reducing power.

It has been recognized since the work of Schön & Engel (1962) that ammonia-oxidizing bacteria are sensitive to near-u.v. light. This results in an absence of nitrification in environments exposed to bright sunlight, such as the photic zone of sea water (Olson, 1981*a*,*b*). Hooper & Terry (1974) studied u.v. irradiation of *N. europaea* in some detail. As the wavelength was lowered, inactivation of ammonia oxidation began at about 430nm. The photosensitivity increased rapidly in the wavelength range 430–400nm, and showed a further slow increase down to about 340nm. Hydroxylamine oxidation was insensitive to light above 400nm, and had a low photosensitivity in the 400–340nm region. No changes in the cellular content of haem proteins could be detected after irradiation, and the nature of the light-absorbing entity remained mysterious. Recovery took a period of hours and required synthesis of fresh protein.

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Experimental

Preparation of cells

Nitrosomonas europaea (A.T.C.C. 19178) was grown and harvested as described by Hyman & Wood (1983). The washed cells were resuspended

at 0.2g wet wt./ml in either 40mM-potassium phosphate buffer, pH7.0, or 50mM-potassium phosphate buffer, pH7.5, as specified below. They were stored at 0°C and used within 24h.

Illumination of cells

The light-source was a C-62 Blak-Ray Trans-illuminator (Ultra-Violet Products, Cambridge, U.K.). The wavelength distribution of the emitted light was investigated with a Farrand spectro-fluorimeter (Farrand Optical Co, New York, NY, U.S.A.). The emission was found to peak at 360nm, with 90% of the emitted light being in the wavelength range 330–390nm. The output power at a distance of 1cm was measured as 24W/m^2, by means of a thermopile (model 14BT; Laser Instrumentation, Chertsey, Surrey, U.K.). In certain experiments a 1cm filter of 90%-saturated KNO_3 solution was placed in a glass dish resting on the transilluminator. This absorbed strongly below 340nm. The cells were diluted with buffer as for resuspension and poured into a conical flask to give a depth of 5mm. The flask was placed 1cm above the surface of the transilluminator. The temperature remained close to ambient.

Assay procedures

NH_4^+-oxidation activity was assayed at 30°C by measuring the rate of O_2 uptake in the presence of a given amount of NH_4^+ (Hyman & Wood, 1983). Hydroxylamine oxidation was assayed in the same way. Bromoethane-oxidation activity was assayed by determining the acetaldehyde production from 2mM-bromoethane during a timed incubation with 600μM-trimethylquinol (trimethylhydroquinone) present to provide reducing power. The reaction was stopped by adding 10μM-allylthiourea. Acetaldehyde was detected by g.l.c. with a Pye–Unicam PU-4500 chromatograph (Pye–Unicam, Cambridge, U.K.) fitted with a flame-ionization detector and a 1.5m column of Tenax GC (80–100 mesh). A sample volume of 5μl was used and an N_2 flow rate of 30ml/min. The injection port was maintained at 150°C and the column at 80°C. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V; Sigma Chemical Co., Poole, Dorset, U.K.) as standard.

Absorption spectroscopy

Difference absorption spectra were recorded with a Pye–Unicam SP.8-200 u.v.-visible spectrophotometer at room temperature, with 1cm-light-path quartz cuvettes. Haemocyanin was from *Limulus polyphemus* haemolymph (type VIII; Sigma Chemical Co., Poole, Dorset, U.K.).

Results and discussion

Photosensitivity and protection from inactivation

Fig. 1 shows the sensitivity of ammonia oxidation to near-u.v. light and relative insensitivity of hydroxylamine oxidation, as reported by Hooper & Terry (1974). Since their study it has become clear that ammonia mono-oxygenase is also capable of bringing about organic oxidations (Drozd, 1980; Hyman & Wood, 1983, 1984*a*). These reactions are all sensitive to the same inhibitors, and are mutually competitive. We have found organic oxidations to be equally sensitive to near-u.v. light, as is shown in Fig. 1 for acetaldehyde production

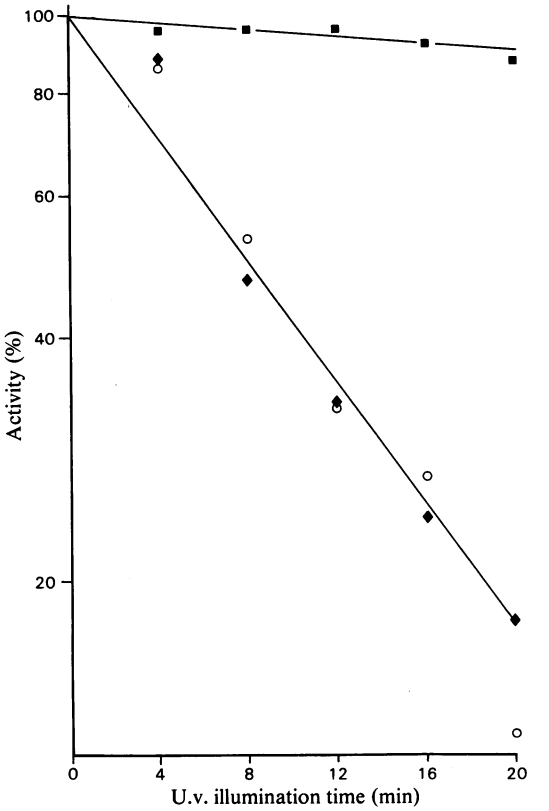


Fig. 1. *Effect of u.v. illumination on rates of NH_4^+, hydroxylamine and bromoethane oxidation by N. europaea cells*

The cells were diluted to 30mg wet wt./ml with 40mM-potassium phosphate buffer, pH7.0, and were subjected to u.v. illumination. At known times samples were withdrawn and stored on ice. Rates of NH_4^+ (◆), hydroxylamine (■) and bromoethane (○) oxidation were determined at 30°C, with 2mM-NH_4Cl, 300μM-hydroxylamine and 2mM-bromoethane respectively. The ordinate gives percentage activity remaining on a logarithmic scale.

from bromoethane. This reaction is believed to take place as follows (Hyman & Wood, 1984*b*):

$$CH_3CH_2Br \xrightarrow{[O]} CH_3CH(OH)Br \xrightarrow{\text{Spontaneous}} CH_3CHO + H^+ + Br^-$$

For Fig. 1 washed cells were illuminated in aerobic suspension, with no added substrate or inhibitor. Fig. 2 shows how alterations to this procedure affected photosensitivity. Any inhibitor or substrate present during illumination was removed by washing before the final assay. It was

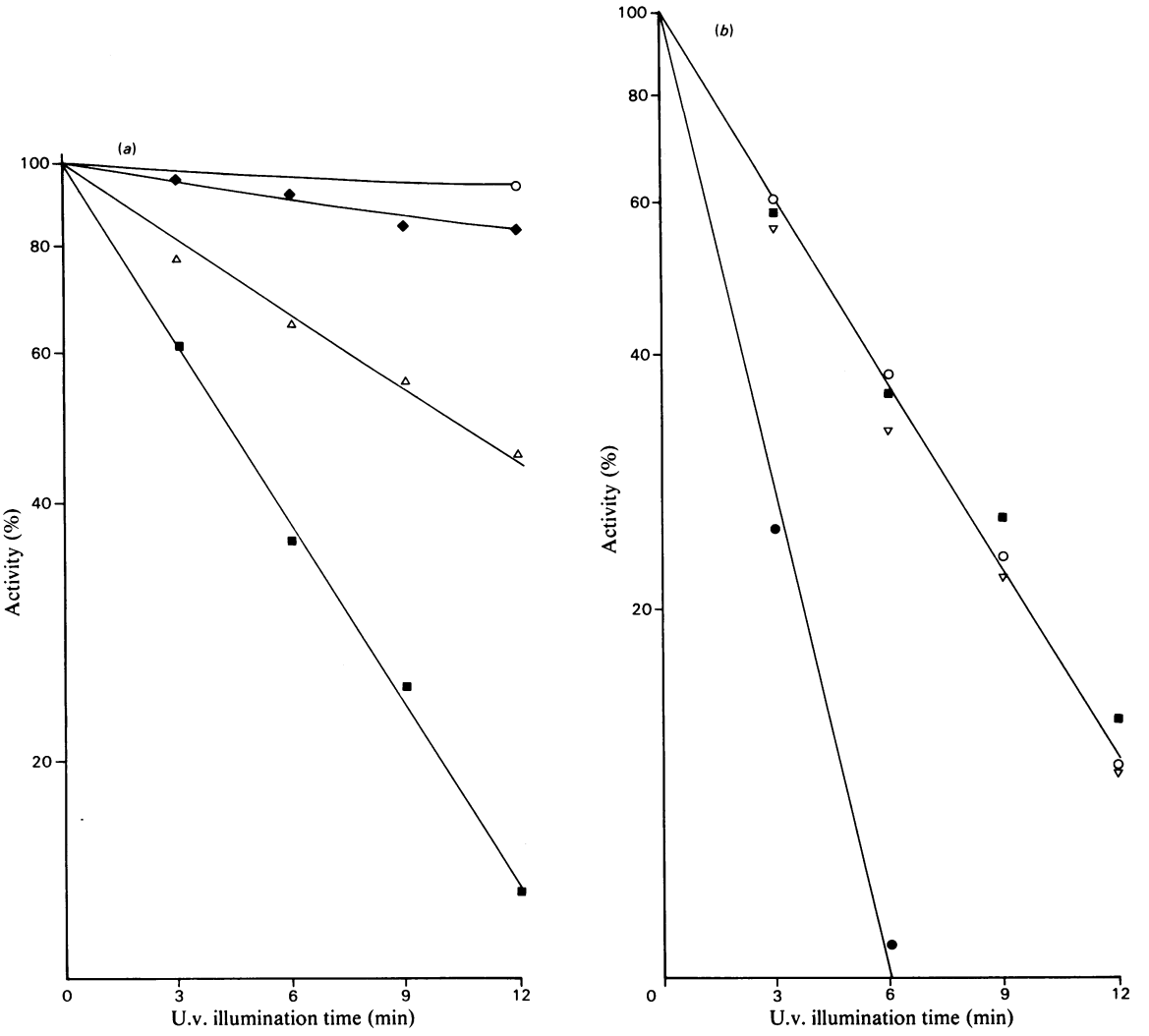


Fig. 2. *Photosensitivity of NH_4^+ oxidation by N. europaea cells under different conditions*

The cells were diluted to 1 mg wet wt./ml (*a*) or 0.5 mg wet wt./ml (*b*) with 50 mM-potassium phosphate buffer, pH 7.5. A 1 cm filter of 90%-saturated KNO_3 solution was employed. For illumination under anaerobic conditions the flask was stoppered and evacuated three times with a water pump, with flushing each time with O_2-free N_2. Samples (1.5 ml) were withdrawn at intervals during illumination. They were spun in a microcentrifuge, and the pellets resuspended in buffer. In the experiment with thiourea present during illumination two further washes were given, with resuspension each time in 1 ml of buffer containing 0.15 mM-NH_4^+. NH_4^+-oxidation activity was determined in a total volume of 1 ml, with 10 mM-NH_4^+ present. For (*a*) the conditions during illumination were as follows: ■, standard procedure; ◆, 5 mM-bromoethane present; △, 20 μM-thiourea present; ○, anaerobic. For (*b*) the following reagents were present during illumination: ■, no additions; ▽, 10 mM-NH_4^+; ○, 1 mM-hydroxylamine; ●, 1 mM-hydrazine.

found that the absence of O_2 was protective. This important feature was reported by Hooper & Terry (1974). The presence of an organic substrate for the mono-oxygenase was also protective. This is shown in Fig. 2(*a*) for bromoethane. In the study by Hooper & Terry (1974) a protective effect of high concentrations of methanol was hard to explain. The strong similarities of the substrate range with that of methane mono-oxygenase make it very probable that methanol is a substrate, although this has not been investigated in detail (see Hyman & Wood, 1984*a*). CO was likewise found by Hooper & Terry (1974) to be protective, and has since been shown to be oxidized to CO_2 (Tsang & Suzuki, 1982).

Thioureas and diethyldithiocarbamate inhibit the mono-oxygenase, probably by chelating copper at the active site, as discussed below (Hooper & Terry, 1973). Fig. 2(*a*) shows that a partial protection was provided by the presence of 20 μM-thiourea during illumination. In another experiment, cells were illuminated for 10 min in the presence of no added inhibitor, 100 μM-thiourea or 50 μM-diethyldithiocarbamate. They were then washed three times, with 50 μM-$CuSO_4$ present in the diethyldithiocarbamate experiment and other conditions as for thiourea in Fig. 2(*a*). Their respective activities were 20, 83 and 60% of the value for unilluminated untreated cells. The protective effect of thiourea, particularly at higher concentrations than are necessary for effective inhibition, was reported by Hooper & Terry (1974).

Fig. 2(*b*) compares the effects of NH_4^+, hydroxylamine and hydrazine on photoinactivation. Hydroxylamine and hydrazine are alternative substrates for hydroxylamine dehydrogenase (Nicholas & Jones, 1960). In addition, hydroxylamine exerts an inhibitory effect on ammonia mono-oxygenase, which is believed to be important in regulating the steady-state hydroxylamine concentration during ammonia oxidation (Hyman & Wood, 1984*b*). A dilute suspension was used with these reductants, to ensure that the rapid rate of respiration did not lead to depletion of O_2. The presence of 10 mM-NH_4^+ or 1 mM-hydroxylamine had no significant effect on the rate of photoinactivation, whereas 1 mM-hydrazine had a potentiating action. These experiments were repeated, and the same results were obtained. By contrast, Hooper & Terry (1974) reported that ammonia and hydroxylamine had a protective effect; they did not try illumination in the presence of hydrazine.

A rapid inactivation was only observed in the presence of O_2 (see Fig. 2*a*). There have been reports of active states of oxygen causing cleavage of polypeptide chains (Creeth *et al.*, 1983). However, u.v. irradiation caused no change in the pattern of bands observed when dodecyl sulphate/polyacrylamide gels were loaded with solubilized cell membranes and subjected to electrophoresis (results not shown).

Spectroscopic changes induced by near-u.v. illumination

Difference spectra were taken with u.v.-treated cells in the sample cuvette and untreated cells as reference. It was found that near-u.v. illumination led to the progressive development of a broad trough in the 350–400 nm region, as shown in Fig. 3. The development of the trough paralleled the destruction of mono-oxygenase activity. There were also minor changes above 400 nm, mostly attributable to cytochromes. The size of the trough was small in relation to total pigment absorption (see Fig. 4), which explains why it escaped notice in earlier studies. However, the trough was reproducibly obtained, and was stable in the dark over a period of at least 1 h.

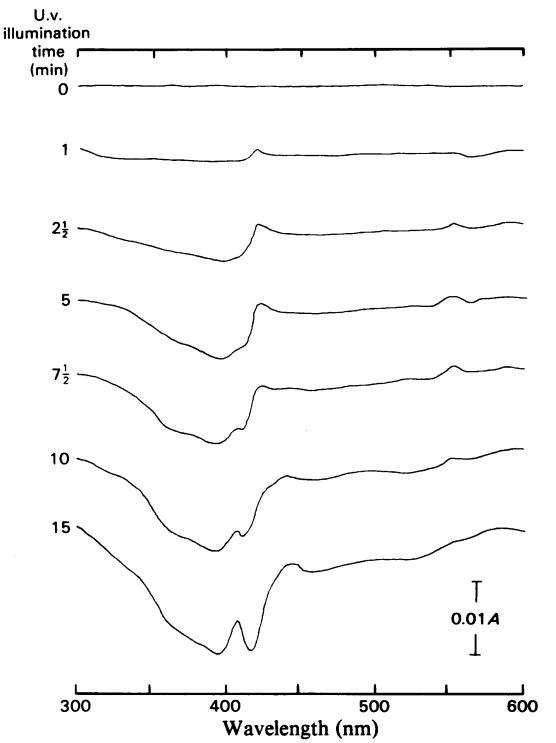


Fig. 3. *Spectroscopic changes in N. europaea cells induced by near-u.v. illumination*

The cells were diluted to 4 mg wet wt./ml with 40 mM-potassium phosphate buffer, pH 7.0, and divided between two 1 cm quartz cuvettes. A baseline was 'memorized' between 300 and 600 nm. The sample cuvette was withdrawn and illuminated on its side at a distance of 1 cm from the transilluminator. Spectra were taken after illumination for the times shown.

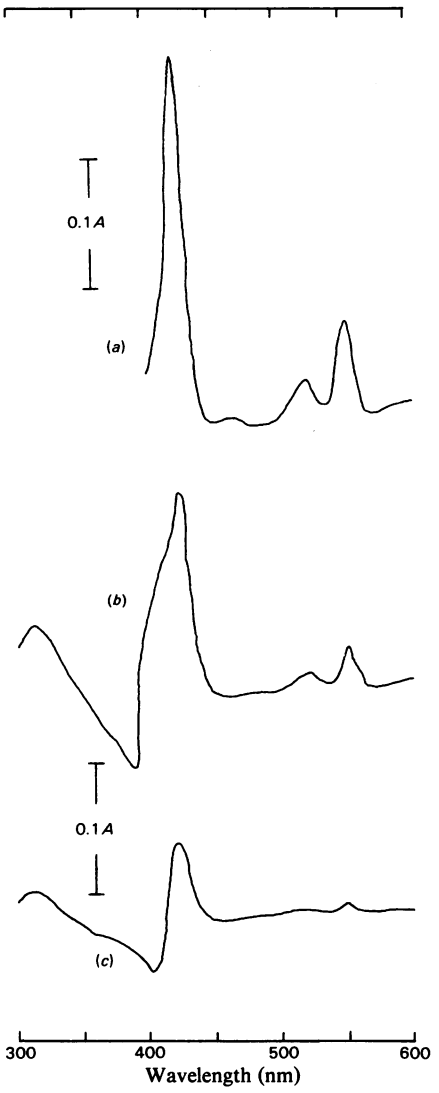


Fig. 4. *Difference spectra of N. europaea cells with dithionite, hydroxylamine and NH_4^+*

The cells were diluted as for Fig. 3. Each spectrum is relative to a 'memorized' baseline. (*a*) Sample plus a few grains of $Na_2S_2O_4$; (*b*) sample plus 500 μM-hydroxylamine; (*c*) sample plus 500 μM-NH_4^+.

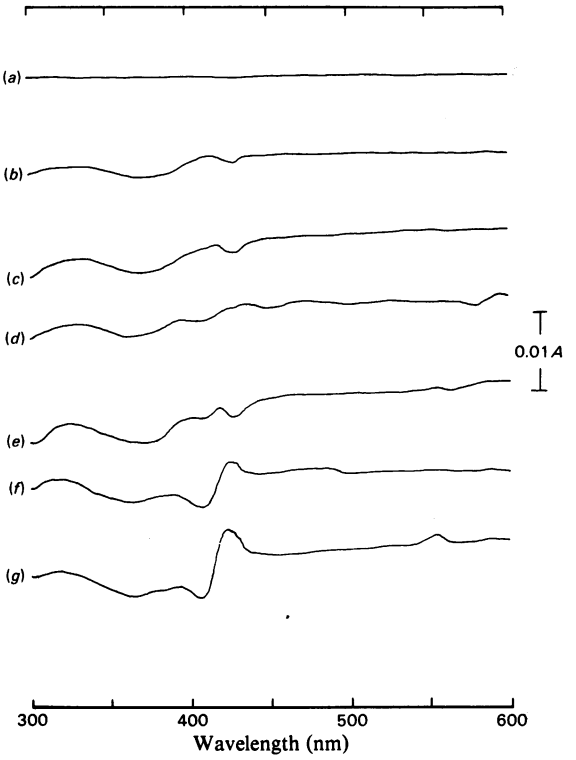


Fig. 5. *Spectroscopic changes in N. europaea cells induced by substrates and inhibitors of the ammonia mono-oxygenase*

The cells were diluted as for Fig. 3. A fresh baseline was 'memorized' for each cuvette filling. (*a*) Baseline; (*b*) sample plus 1 mM-bromoethane; (*c*) sample plus 225 μM-ethylene; (*d*) sample plus 5 mM-methanol; (*e*) sample bubbled briefly with CO; (*f*) sample plus 60 μM-acetylene, after a 5 min incubation; (*g*) sample plus 10 μM-allylthiourea, after a 5 min incubation. For (*c*) and (*f*) the sample cuvette was filled to the brim and stoppered (Suba-Seal; Gallenkamp, London, U.K.). The gas was added from an aqueous solution equilibrated at 0.1 MPa (1 atm) pressure, at 0°C, assuming solubilities as stated in Washburn (1928).

In view of the small size of the changes relative to total pigment absorption, it was not feasible to illuminate in the presence of a substrate or inhibitor, wash the cells, and take spectra relative to an untreated reference.

Other agents causing comparable spectroscopic changes

In aerobic resting cells the cytochromes are predominantly oxidized. Addition of hydroxylamine caused substantial cytochrome reduction (Fig. 4*b*), which would mask any near-u.v. absorption changes comparable with those just described. Addition of NH_4^+ also led to appreciable cytochrome reduction (Fig. 4*c*), because ammonia oxidation implies a continual generation of hydroxylamine. The results with organic substrates and inhibitors of the mono-oxygenase were more interesting.

The spectroscopic changes induced by four organic substrates, bromoethane, ethylene, methanol and CO, are shown in Fig. 5. [The status of methanol as a substrate was discussed above; for

the others see Tsang & Suzuki (1982) and Hyman & Wood (1984*a*,*b*).] All gave rise to a broad trough centred near 370nm, in difference spectra relative to an untreated reference. A similar near-u.v. trough was obtained on addition of inhibitors. Acetylene (Fig. 5*f*) has been shown to cause irreversible inactivation of the enzyme (Hynes & Knowles, 1982) and is believed to act as a suicide substrate (M. R. Hyman & P. M. Wood, unpublished work). A similar spectrum was generated with a reversible inhibitor, allylthiourea (Fig. 5*g*).

The 400–450nm region showed opposing effects with organic substrates and inhibitors. With several substrates a small peak developed in difference spectra, with a trough on its long-wavelength side. Conversely, acetylene and allylthiourea both led to a complementary trough, plus a peak on the long-wavelength side. In our experience, endogenous respiration of washed cells is always inhibited by 10–30% on addition of thiourea, implying that a low rate of ammonia oxidation is taking place, perhaps as a result of breakdown of protein. The simplest interpretation of the 400–450nm spectral changes is that the degree of oxidation of a certain cytochrome depends on the rate of electron flow to the mono-oxygenase. This electron flow is increased by organic substrates and decreased by inhibitors; hence the cytochrome becomes respectively more oxidized or more reduced.

A photosensitive oxygenated state of a copper enzyme?

The results presented above show that illumination of resting cells with near-u.v. light inactivates ammonia mono-oxygenase and leads to a decrease in absorption in the 350–400nm region. Organic substrates and reversible inhibitors of the mono-oxygenase protect against photoinactivation. Organic substrates and inhibitors also bring about an absorption decrease in the 350–400nm region. The simplest explanation is that the photosensitive state of ammonia mono-oxygenase has a broad near-u.v. absorption band, and this absorption is removed, or at least diminished, by organic substrates and inhibitors.

In quantitative terms the trough induced by u.v. illumination was larger than that developed by substrates or inhibitors, and had an absorbance minimum at a slightly longer wavelength: for u.v.-irradiated cells, after 10min, $\Delta\varepsilon = 2.2 \times 10^{-2}$ l·(g of protein)$^{-1}$·cm^{-1}, minimum at 390nm; for ethylene-treated cells, $\Delta\varepsilon = 1.3 \times 10^{-2}$ l·(g of protein)$^{-1}$·cm^{-1}, minimum at 378nm. These differences may reflect residual absorption in the presence of substrates and inhibitors. Alternatively, they may be caused by secondary effects of u.v. light, since the photoinactivation is not completely specific.

Ammonia mono-oxygenase has not yet been purified and has not previously been assigned any spectrum, absorption or otherwise. As a consequence any discussion of the u.v.-sensitive chromophore must be of a speculative nature. The enzyme is known to be inhibited by reagents that have a high affinity for cuprous copper, for instance thioureas, cyanide, diethyldithiocarbamate and αα′-dipyridyl (Hooper & Terry, 1973). Most copper enzymes that have a function other than electron transfer are sensitive to thioureas, examples being tyrosinase (Prabhakaran *et al.*, 1968) and dopamine β-mono-oxygenase (Johnson *et al.*, 1969). The mono-oxygenase has a specificity for organic substrates very like that for the membrane form of methane mono-oxygenase (Hyman & Wood, 1983, 1984*a*). Stanley *et al.* (1983) have shown that synthesis of this form of methane mono-oxygenase (as opposed to a soluble form) requires the addition of copper to the growth medium. Copper is likewise required by *Nitrosomonas* (Loveless & Painter, 1968). One may conclude that ammonia mono-oxygenase is in all probability a copper enzyme.

Two copper-containing mono-oxygenases have been extensively studied, dopamine β-mono-oxygenase and tyrosinase. Dopamine β-mono-oxygenase is sensitive to anionic copper-ion chelators such as bathocuproinedisulphonate, which have little effect on *N. europaea* (Hooper & Terry, 1973; Rosenberg & Lovenberg, 1980). It also lacks significant absorption bands above 300nm (Friedman & Kaufman, 1965). Tyrosinase is much closer to ammonia mono-oxygenase in its sensitivity to chelators (Solomon, 1981). Furthermore, it is sensitive to near-u.v. irradiation and has a prominent near-u.v. absorption band in its oxygenated state (Solomon, 1981). These analogies are pursued further in the following sections.

Comparison with tyrosinase and haemocyanin

Tyrosinase is the generic name for a group of copper proteins that typically have both catechol oxidase (EC 1.10.3.1) and monophenol mono-oxygenase (EC 1.14.18.1) activities (Dixon & Webb, 1979; Lerch, 1981). The latter reaction can be written as follows:

$$RH + XH_2 + O_2 \rightarrow ROH + X + H_2O$$

For example, hydroxylation of tyrosine (RH) leads to formation of dopa (3,4-dihydroxyphenylalanine) (ROH). This acts as an internal reductant (i.e. as XH_2), yielding dopaquinone (X) as final product. (Ammonia mono-oxygenase needs an external reductant, the normal ultimate donor of electrons being hydroxylamine or endogenous substrates.)

The active site of tyrosinase contains two copper atoms in close interaction, and resembles closely that of haemocyanin, an O_2-binding protein found in arthropods and molluscs (Solomon, 1981). For both tyrosinase and haemocyanin three states are well characterized. These have been labelled Deoxy, Oxy and Met, by analogy with haemoglobin and myoglobin (Lerch, 1981; Solomon, 1981). The chemical configurations and spectroscopic properties of these states are summarized in Table 1. The Deoxy state has cuprous copper, which has a filled *d*-shell, and has no detectable absorption above 300nm. Reaction with Cu(I) ligands such as thiourea does not affect this closed-shell configuration, and such complexes normally only have weak absorption above 300nm (Rombauts, 1968). Oxygen is bound reversibly in the form of peroxide (O_2^{2-}), with concomitant oxidation of the copper atoms to Cu(II) (Solomon, 1981). This Oxy state has a strong absorption band centred near 345nm, with a half-width of about 50nm. The peak absorbance lies at 345nm for *Neurospora* and mushroom tyrosinase and *Cancer* (crab) haemocyanin, and at 340nm for *Busycon* (mollusc) haemocyanin (Jolley *et al.*, 1974; Lerch, 1976; Freedman *et al.*, 1976). The Met state, which also contains Cu(II), has much weaker near-u.v. absorption bands.

The oxy complexes of both proteins are sensitive to near-u.v. light. This is explained by the fact that the 345nm absorption band corresponds to a charge-transfer transition (Solomon, 1981). In the excited state the σ-bond between oxygen and copper is eliminated, and the peroxide ion is converted into superoxide (O_2^-). Dissociation can easily occur, and results in a free superoxide ion being produced at the active site.

Fig. 6 shows an absorption spectrum for oxyhaemocyanin, and also shows how the absorption is affected by u.v. irradiation. The trough observed in difference spectra, irradiated versus unirradiated, can be compared with the troughs presented for *N. europaea* in Figs. 3 and 4.

A speculative model for the catalytic cycle

The results presented above point to a light-sensitive oxygenated state of ammonia mono-oxygenase, with a broad near-u.v. absorption band having a similar half-width to that of tyrosinase or haemocyanin, but centred at a slightly longer wavelength. As a working hypothesis, a model for the catalytic cycle can be based on that for tyrosinase (Lerch, 1981). The mono-oxygenase activity of tyrosinase involves O_2 binding to the Deoxy state. This gives the Oxy state, which is the active form for the mono-oxygenase reaction. One

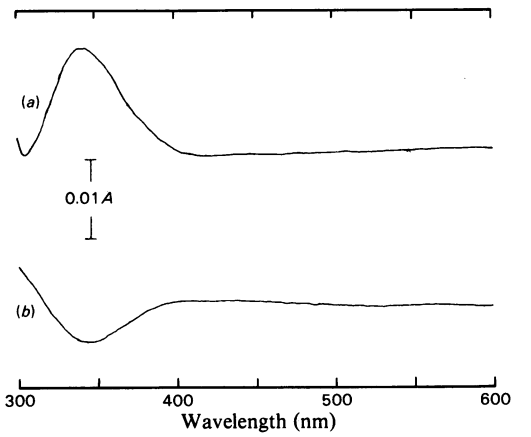


Fig. 6. *Haemocyanin spectra*

Haemocyanin was dissolved in 40mM-potassium phosphate buffer, pH7.0, at 1.5mg/ml. (*a*) Sample untreated (i.e. oxyhaemocyanin), reference bubbled with CO, then stoppered. (*b*) Sample illuminated for 30min as described in Fig. 3, reference untreated. In (*a*) the CO complex was used as reference because the high M_r of haemocyanin leads to appreciable light-scattering. The CO complex contains cuprous copper (unlike the oxy complex); the only significant absorption in the 300–600nm region is from a weak charge-transfer band, $\varepsilon_{310} = 1.8 mM^{-1} \cdot cm^{-1}$ (Solomon, 1981).

Table 1. *Principal states of haemocyanin and tyrosinase*

Data are taken from Lerch (1981) and Solomon (1981).

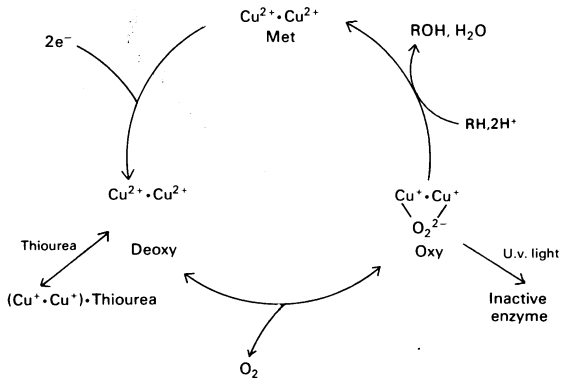
State	Configuration	Absorption bands, 300–800nm	ε for Cu–Cu pairs ($mM^{-1} \cdot cm^{-1}$)
Deoxy	Cu(I)–Cu(I)	No significant absorption	
Oxy	Cu(II)–Cu(II)	340–345nm, $O_2(\pi_\sigma^*) \rightarrow Cu(d_{x^2-y^2})$	18–20
	O_2^{2-}	570–600nm, $O_2(\pi_v^*) \rightarrow Cu(d_{x^2-y^2})$ plus weak bands as for Met	1–1.2
Met	Cu(II)–Cu(II)	Several weak $d \rightarrow d$ and ligand→Cu bands:	
		300–400nm	<1.5
		400–800nm	<0.3

O atom is incorporated into the substrate, one is reduced to water, and the enzyme is left in the oxidized Met state. A two-electron reduction regenerates the original Deoxy form. It should be noted that the conversion of the Deoxy into the Oxy form is freely reversible. Scheme 1 presents an analogous catalytic cycle for ammonia mono-oxygenase.

In washed cells there is always a significant endogenous respiration. The fact that organic substrates can be hydroxylated without added reductant implies that conversion of the Met form into the Deoxy form can be brought about by endogenous reducing power (cf. Hyman & Wood, 1983). Thus aerobic washed cells will contain the enzyme predominantly in the Oxy state. This is the form that absorbs u.v. light and is subject to photo-inactivation. Under anaerobic conditions the bound O_2 dissociates, leaving the enzyme in the u.v.-insensitive Deoxy state. Chelating agents for Cu(I) trap the enzyme in a light-insensitive cuprous complex. Organic substrates protect by bringing about a rapid conversion of the Oxy form into the Met form.

The potentiation of photoinactivation by hydrazine can also be understood. As was mentioned above, part of the endogenous respiration is attributable to ammonia oxidation. As a result the enzyme will be turning over in washed cells, and will not be exclusively in the Oxy state. Hydrazine, by acting as an external source of reductant, will increase the rate of reduction of the Met form. This will increase the proportion in the light-sensitive Oxy state.

The effects of added ammonia or hydroxylamine are harder to predict. Ammonia reacts with the Oxy state, generating the Met state and hydroxylamine. Further oxidation of this hydroxylamine supplies electrons for reduction of the Met form to the Deoxy form. Thus the enzyme is in a state of rapid turnover. Conversely, added hydroxylamine has an inhibitory effect on turnover of the enzyme, as explained above. These reagents gave rise to the only important discrepancies between our results and those reported by Hooper & Terry (1974). With both of them the precise proportion of the Oxy state may depend critically on the state of the cells.



Scheme 1. *A possible catalytic cycle for ammonia mono-oxygenase*

It should be emphasized that the cycle presented in Scheme 1 is of a speculative nature. We have assumed the presence of two copper atoms because of certain similarities with tyrosinase, and because there is no precedent for a single copper at the active site of a mono-oxygenase. However, the example of cytochrome *P*-450 shows that a single metal atom can have mono-oxygenase activity. The photoinactivation of tyrosinase has been attributed to liberation of superoxide at the active site, as explained above. Copper enzymes can also be sensitive to H_2O_2, as discussed by Fielden & Rotilio (1984) for Cu/Zn superoxide dismutase. A third possible active state of oxygen is the hydroxyl radical.

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