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INHIBITION OF RESPIRATION AND DESTRUCTION OF CYTOCHROME A_3 BY LIGHT IN MITOCHONDRIA AND CYTOCHROME OXIDASE FROM BEEF HEART

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SUMMARY

Irradiation of beef-heart mitochondria and of cytochrome oxidase purified from beef-heart mitochondria with blue light inhibited electron transport from substrate (succinate for the mitochondria and reduced cytochrome *c* for the cytochrome oxidase) to O_2. The irradiation treatment also destroyed cytochrome a_3 as assayed by the absorption band for the reduced cyanide–cytochrome a_3 complex at 587 nm in the low-temperature absorption spectrum. Irradiation under anaerobic conditions was not inhibitory. Cytochrome a_3 was protected against photodestruction if cyanide was present during the irradiation.

INTRODUCTION

The investigation of the effects of light on the respiratory electron transport chain in beef-heart mitochondria and cytochrome oxidase purified from those mitochondria reported here derived from an observation that blue light inhibited growth in a colorless alga, *Prototheca zopfii*[1]. In subsequent work it was found that blue light inhibited respiration of both actively growing and starved cultures of the alga and that the inhibition of respiration was concomitant with photodestruction of cytochrome a_3. The photoinhibition of growth and cell division was assumed to be a consequence of the photoinhibition of respiration. A cytochrome oxidase-dependent photoinhibition of respiration was also found in yeast[4] where both cytochromes *a* and a_3 were destroyed by blue light. In the work reported here the effect of light on respiratory electron transport was studied with beef-heart mitochondria. In both cases irradiation with blue light inhibited electron transport from substrate (succinate for beef-heart mitochondria or reduced cytochrome *c* for cytochrome oxidase) to O_2 and destroyed cytochrome a_3 but not cytochrome *a*. In all cases studied thus far, O_2 was required during the irradiation period for photoinhibition of electron transport and photodestruction of respiratory pigments and cyanide protected cytochrome a_3 against photodestruction when O_2 was present.

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METHODS

Beef-heart mitochondria and cytochrome oxidase purified from beef heart were prepared in Dr. Y. Hatefi's laboratory at Scripps Clinic and Research Foundation according to methods described previously[5,6]. About 30 mg of the mitochondria pellet were suspended in 5 ml of a medium containing 0.05 M Tris–HCl (pH 8.0) and 0.6 M sucrose. This stock suspension was further diluted 20–40-fold with the same medium to a standard concentration (the absorption band of reduced cytochrome oxidase ($A_{607\ nm} - A_{630\ nm}$) at room temperature was 0.01/cm) for the irradiation treatments and the subsequent measurements. The cytochrome oxidase pellet, approx. 1 g containing about 26 mg of protein, was suspended in 2 ml of a medium containing 0.05 M Tris–HCl (pH 8.0) 1 mM histidine and 0.66 M sucrose. The stock suspension of the oxidase was diluted with the oxidase medium 20-fold for irradiation treatments and an additional 33-fold for activity measurements or 5-fold for spectroscopy.

The suspensions of mitochondria and cytochrome oxidase were irradiated with a 1000-W high-pressure mercury lamp (G.E. AH-6) through a blue glass filter (Corning 5562 ($2 \cdot 10^6$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}). The suspensions were stirred with a magnetic stirrer in a 5-ml rectangular cuvette (1-cm optical path) in a water bath kept near 0° during the irradiation period. For anaerobic experiments with cytochrome oxidase the suspension was made up with deaerated medium and Ar was bubbled slowly through the suspension in the cuvette for 2 h before and during the irradiation. The bubbling had to be quite slow to avoid foaming.

Respiratory activity was measured as the rate of O_2 uptake at 27° with a Clark-type oxygen electrode (Yellow Springs Instrument Co., Model 4004) in a 3.3-ml cuvette. 50 μl of 0.1 M succinate were added to the cuvette as substrate for the mitochondria; 50 μl of 0.1 M ascorbate and 100 μl of 1 mM cytochrome *c* were used with the cytochrome oxidase.

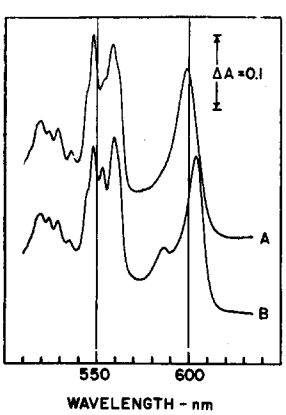
Absorption spectra were measured with a single-beam spectrophotometer (Tungsten lamp, Cary Model 14 Monochromator, EMI 9558C phototube and logarithmic photometer) on line with a digital computer (Digital Equipment Corp. PDP 8/I) *via* a 12-bit analog-to-digital converter (Digital Equipment Corp. AF-01). The single-beam absorption spectra of the samples and of a light-scatter reference were stored in the memory core of the computer and displayed on an X–Y recorder as the difference between the sample and the reference. 1-ml samples were placed in a vertical path cuvette which gave a sample depth of 6 mm and were frozen to −196° in an open-topped Dewar as described previously[7]. The light scatter in the frozen samples intensified the absorption bands about 30-fold (by increasing the optical path length)[8].

RESULTS

It was found previously with *P. zopfii* that cytochromes *a* and a_3 could be resolved spectrally in the α-band region by reducing the cells in the presence of cyanide and methanol[2,9]. Methanol caused the absorption maximum of reduced cytochrome *a* to shift from 598 to 603 nm and permitted dithionite to reduce the cyanide–cytochrome a_3 complex to give a 595-nm absorption band. In the absence of methanol, dithionite did not reduce the cyanide–cytochrome a_3 complex. Fig. 1

shows a similar effect with beef-heart mitochondria. The absorption spectrum of mitochondria reduced with dithionite, Curve A, shows cytochrome *c* with a maximum at 548 nm and a shoulder at 545 nm, cytochrome c_1 as a shoulder at 554 nm, two *b*-type cytochromes, one with a maximum at 559 nm, the other as a shoulder at about 562 nm, and cytochrome oxidase with a maximum at 599 nm. Reduction of the mitochondria by dithionite in the presence of methanol (2.5 %) and cyanide (2.5 mM) resulted in an absorption band at 604 nm for cytochrome *a* and a small band at 587 nm for the reduced cyanide-cytochrome a_3 complex. Reduction by dithionite in the presence of cyanide in the absence of methanol, or with methanol in the absence of cyanide, did not give the 587-nm absorption band. The 587-nm band found in the presence of cyanide, methanol and dithionite has been used as a spectral assay for cytochrome a_3. Methanol also induced changes in the α-bands of the *b*- and *c*-type cytochromes such that the absorption band of cytochrome c_1 was more prominent in the presence of methanol.

O_2 uptake by the mitochondria with succinate as substrate was inhibited by irradiation with blue light ($2 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) (Fig. 2). Absorption spectra of the mitochondria measured in the presence of dithionite, cyanide, and methanol to resolve cytochromes *a* and a_3 (Fig. 3) showed that the irradiation treatment altered cytochrome a_3 to the extent that the 587-nm band was lost. Cytochrome a_3 was protected if the irradiation was carried out in the presence of cyanide. We will refer to photodestruction of cytochrome a_3 without implying the nature of the destruction. Photodestruction of cytochrome a_3 was also demonstrated by comparing the CO photodissociation difference spectra of nonirradiated and irradiated mitochondria. Nonirradiated mitochondria showed a typical CO difference spectrum for cytochrome a_3 which was not found with irradiated mitochondria. The results were essentially the same as the CO difference spectra of nonirradiated and irradiated cells of *P. zopfii*[2]. In yeast, photodestruction of cytochromes *a* and a_3 was accompanied by a partial loss of cytochrome *b* (ref. 4) and in *P. zopfii*, which contains two



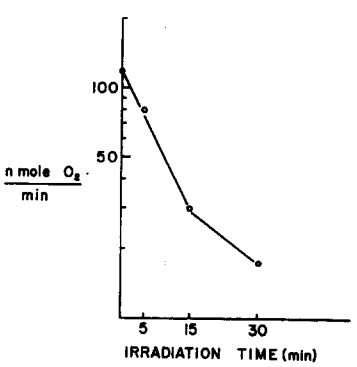


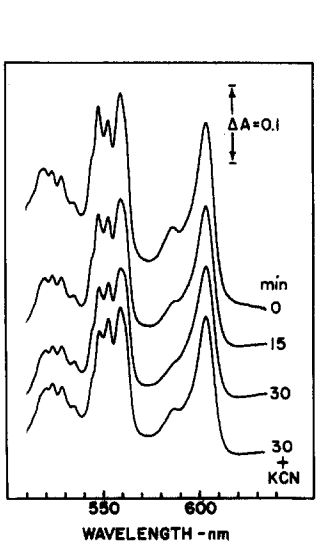
Fig. 1. Absorption spectra of reduced beef-heart mitochondria at 77°K. A, reduced by dithionite; B, reduced by dithionite in the presence of 2.5 % methanol and 2.5 mM KCN.

Fig. 2. Rate of O_2 uptake by beef-heart mitochondria at 27° with 1.5 mM succinate as substrate as a function of the time of irradiation. Approx. 0.66 mg of the mitochondria pellet was suspended in 3.3 ml of the reaction medium (pH 8.0) containing 0.05 M Tris–HCl and 0.6 M sucrose

c-type and three *b*-type cytochromes, irradiation destroyed one of the *c*-type and one of the *b*-type along with cytochrome a_3 (refs. 2, 3).

The cytochrome oxidase purified from beef-heart mitochondria was also examined. Reduction of the oxidase preparation with dithionite (Fig. 4A, lower curve) resulted in the main absorption band at 602 nm attributable primarily to cytochrome *a* and a very small band at 577 nm apparently due to cytochrome a_3. The 577-nm band was absent when the oxidase was reduced in the presence of cyanide (Fig. 4A, middle curve). Reduction in the presence of both cyanide and methanol gave a band at 587 nm attributable to the reduced cyanide–cytochrome a_3 complex (Fig. 4A, upper curve) in addition to the main band for cytochrome *a*. Irradiation of the oxidase preparation with blue light ($2 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) for 1 h (Fig. 4B) eliminated the 577-nm band found after dithionite reduction and the 587-nm band found when reduction occurred in the presence of cyanide and methanol. The protection of cytochrome a_3 against photodestruction by cyanide could also be demonstrated with the cytochrome oxidase preparations under the same conditions used with the mitochondria for Fig. 3.

The activity of the purified oxidase preparation was measured as the rate of O_2 uptake with reduced cytochrome *c* as substrate. Irradiation of the oxidase with blue light ($2 \cdot 10^6$ ergs·cm^{-2}·sec^{-1}) under aerobic conditions resulted in inhibition of the oxidase activity (Fig. 5). Irradiation under anaerobic conditions (Fig. 5) was almost without effect. (In previous work with *P. zopfii*[3] and yeast[4] irradiation under anaerobic conditions had no effect on the respiratory capacity or the cytochrome compostion of the cells.) The small effect of irradiation under anaerobic conditions



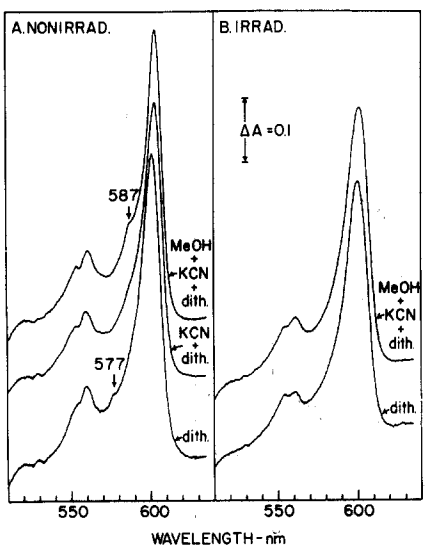


Fig. 3. Absorption spectra of beef-heart mitochondria in the presence of dithionite, 2.5 mM KCN and 2.5 % methanol at 77°K. The mitochondria were irradiated 0, 15, and 30 min in the absence or presence of 1 mM KCN as indicated.

Fig. 4. Absorption spectra of reduced cytochrome oxidase at 77°K. A, non-irradiated; B, after 1 h irradiation. Cytochrome oxidase was reduced with dithionite, with dithionite in the presence of 2.5 mM KCN or by dithionite in the presence of 2.5 mM KCN and 2.5 % methanol as indicated.

was probably due to a lack of complete anaerobiosis. The cytochrome oxidase preparation could not be purged vigorously with Ar because of foaming.

The cytochrome oxidase when suspended in the buffer medium is in the oxidized state and remains oxidized even under anaerobic conditions. The solid curve in Fig. 6 is the low-temperature absorption spectrum of the cytochrome oxidase after bubbling with Ar for 2 h. The dashed curve is the absorption spectrum of the same sample after thawing, adding dithionite and refreezing. Thus, the O_2 requirement for photoinhibition is a requirement for molecular O_2 and not just a requirement for the oxidized state.

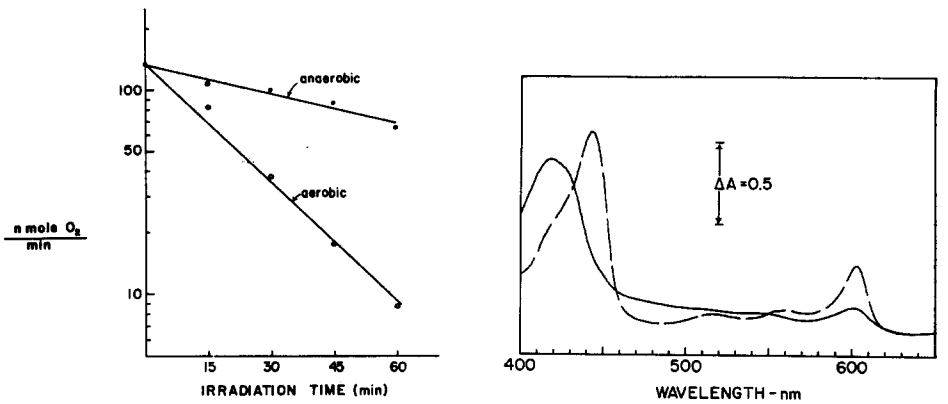


Fig. 5. Rate of O_2 uptake by cytochrome oxidase (20 μg protein/per ml) at 27° with 30 μM cytochrome *c* and 1.5 mM ascorbate as substrate as a function of the time of irradiation under aerobic or anaerobic conditions as indicated. Reaction mixture (pH 8.0) contained 0.05 M Tris–HCl, 1 mM histidine and 0.66 M sucrose.

Fig. 6. Absorption spectra of cytochrome oxidase at 77°K (——) after bubbling with Ar 2 h. — —, same sample, after thawing, adding dithionite and refreezing.

DISCUSSION

The action of light on purified cytochrome oxidase preparations indicated that the destruction of cytochrome a_3 and the resulting inhibition of respiratory activity was due to direct absorption of light by the Soret band of cytochrome a_3. With yeast[4] prolonged irradiation with wavelengths longer than 480 nm had little effect on the cytochrome system whereas shorter wavelengths were effective in destroying cytochrome oxidase and a part of the cytochrome *b*. A crude action spectrum with three wavelengths, 365, 405, and 436 nm, indicated that both 405 and 436 nm were more effective than 365 nm in destroying cytochrome oxidase while 365 nm was the most effective of the three wavelengths for inhibiting exogenous respiration. Apparently the action of near ultraviolet in yeast also involved the inactivation of a quinone in the electron transport chain as well as cytochrome oxidase. The action of blue light, however, was mediated *via* the Soret band of cytochrome oxidase.

Cytochrome a_3 was in the oxidized form during irradiation either in aerated

cultures of starved cells[3,4] or in stirred suspensions of mitochondria or the purified oxidase preparations and O_2 was required for photodestruction. The protection of cytochrome a_3 by cyanide further indicated that the mechanism of photodestruction involves a close association between O_2 and Fe^{3+} of cytochrome a_3. In yeast where both cytochromes *a* and a_3 were destroyed by light similar mechanisms appear to be involved with both pigments since cytochrome *a* was protected against photodestruction by azide, analogous to the protection of cytochrome a_3 by cyanide.

The sensitivity of cytochrome oxidase purified from beef-heart mitochondria to light suggests that photoinhibition of respiration is a general phenomenon in aerobic organisms. The generality of the phenomenon was also indicated by finding a cytochrome oxidase-dependent photoinhibition of respiration in the two organisms examined thus far; *Saccharomyces cerevisiae*, a fungus and *P. zopfii*, a colorless alga with a cytochrome composition typical of higher plants[9]. In both yeast and *P. zopfii* the photoinhibition of respiration is accompanied by an inhibition of protein synthesis and of growth (*i.e.* cell division)[1,3,10]. The intensity of light on sunny days is great enough to markedly inhibit respiration over the duration of the light period. The consequences of such a diurnal variation of respiration on cellular control mechanisms may be considerable especially since organisms have evolved under such an influence.

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