PHOTOINHIBITION OF RESPIRATION IN BACTERIA AND THE CYANOPHYCEA *VITREOSCILLA STERCORARIA*

HELGA NINNEMANN

Institut für Chemische Pflanzenphysiologie, 74 Tübingen, W. Germany

Received 25 May 1972

1. Introduction

Photoinhibition of respiration caused by destruction of cytochrome oxidase has been shown in various higher organisms like the colorless green alga *Prototheca zopfii* [1,2], yeast [3], and beef heart mitochondria [4]. This phenomenon seems to be ubiquitous in colorless cells and not restricted to higher organisms. It is reported here that the respiratory activity of bacteria and of a bluegreen alga as well is impaired by blue light.

2. Materials and methods

Pseudomonas fluorescens, Bacillus subtilis, and *Escherichia coli* were cultured sterilely in liquid complete media (0.5% Difco-bactopeptone, 0.3% Difco-beef extract, 0.25% NaCl). *Vitreoscilla stercoraria* (stock culture from the algal collection Göttingen) was grown in darkness under sterile conditions in a liquid medium containing 0.25% bactopeptone (Difco) and 0.25% beef extract. Before irradiation this culture was washed twice with a starvation medium (3 g KH_2PO_4, 3 g K_2HPO_4, 1 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 0.5 g Na-citrate at 1000 ml, pH 6.8) and irradiated in this medium. *Vitreoscilla* could be kept in the starvation medium for more than a day without loss of respiratory capacity. Peptone and beef extract were added as substrate for oxygen measurements. In *Vitreoscilla* respiration recovery was followed for 6–8 hr after irradiation and addition of substrate before bacterial contamination began to interfere.

As light source a XBO 501 Osram Xenon lamp of 450 W was used together with a blue Corning filter No. 5562 and a Schott cut-off filter KV 393 so that only wavelengths >400 nm were transmitted. A waterfilter of 6 cm was placed between lamp and sample cuvette. Oxygen uptake was measured in dark controls or after irradiation with a Yellow Springs Clark-type oxygen electrode (YSI 4004).

3. Results and discussion

Cytochrome oxidase was shown before to be the light absorbing pigment for the photoinhibition of respiration in higher organisms [4]. So it was of interest to measure the respiratory activity of irradiated lower organisms and of those lacking cytochrome oxidase. The respiration of *P. fluorescens, B. subtilis,* and *E. coli* was inhibited by blue light as shown in fig. 1. The light effect was observed irrespective of the terminal oxidase being an *a*-type or *b*-type cytochrome: for *Pseudomonas* cytochrome a_2, but probably no cytochrome a_3 [5] is reported as electron donor for oxygen, for *B. subtilis* cytochrome *a* [5], but apparently no cytochrome a_3 [6] and for *E. coli* cytochrome *o* (or under certain growth conditions possibly cytochrome *o* and cytochrome a_2 [7]) are described in the literature. In our experiment, *P. fluorescens* and *B. subtilis* (*a*-type terminal oxidases) turn out to be somewhat more light-sensitive than *E. coli* with its *b*-type cytochrome *o* as terminal electron donor.

Viability was checked in *Pseudomonas* after a 60 min irradiation and found to be unaffected by the treatment. Also Rubenstein [8] reported a decrease of respiratory activity after irradiating *Sarcina lutea*

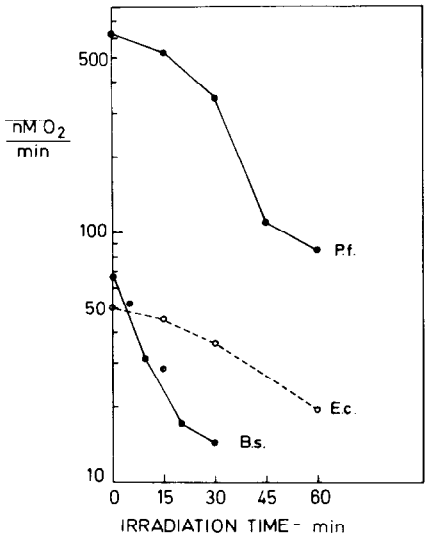

Fig. 1. Effect of light on respiration of bacteria irradiated with 2.5×10^6 ergs cm^{-2} sec^{-1} blue light.
(●–●–●) *P. fluorescens;* (○–○–○) *E. coli;* (○–○–○) *B. subtilis.*

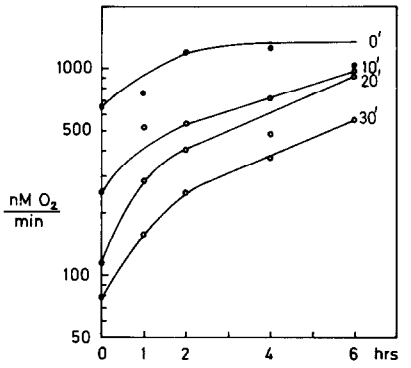

Fig. 2. Effect of light on respiration and respiratory recovery of *Vitreoscilla stercoraria* irradiated with 1.8×10^6 ergs cm^{-2} sec^{-1} blue light ($\lambda \geq 400$ nm) from a 450 W Xenon lamp with Corning filter No. 5562 and Schott KV 393 cut-off filter. Abscissa: time (hr) after addition of substrate to the irradiated samples and dark control.(●–●–●) Dark control; (○–○–○) samples irradiated 10', 20', and 30' resp.

with white light without loss of viability.

The colorless blue-green alga *Vitreoscilla stercoraria* (often also classified as Flexibacterium) has cytochrome *o* as the terminal oxidase [9]. A high sensitivity of *Vitreoscilla* towards blue light was observed. Addition of substrate to the starvation medium after the light treatment leads to a recovery of respiratory activity (fig. 2) as it was found also in yeast [10]. In the case of *Vitreoscilla*, however, it could – in contrast to the experiments with yeast [10] – not be proven that the recovery occurred directly in the irradiated cells, as the added substrate allows cell division.

The high light intensities used are no precondition for a successful photoinhibition of respiration. An irradiation of 5 hr with 10^5 ergs cm^{-2} sec^{-1} of blue light diminished the respiratory rate in starved *P. fluorescens* by 95%. A 5 days' exposure of *Vitreoscilla* in starvation medium to daylight (12 hr) of moderate intensity (about 10^4 ergs cm^{-2} sec^{-1} *white* light) suppressed respiration by 25%.

The reported data emphasize the previous conclusion that visible light of high as well as of moderate intensity generally impairs the respiratory chain in colorless organisms without affecting their viability. This phenomenon was found in bacteria, algae, yeast and animal cells. Irradiated cells in starvation medium arrest their growth and development but can resume synthetic processes upon addition of substrate.

Acknowledgement

I gratefully acknowledge the financial support by the Deutsche Forschungsgemeinschaft.

References

[1] B. Epel and W.L. Butler, Science 166 (1969) 621
[2] B. Epel and W.L. Butler, Plant Physiol. 45 (1970) 728.
[3] H. Ninnemann, W.L. Butler and B. Epel, Biochim. Biophys. Acta 205 (1970) 499.
[4] H. Ninnemann, W.L. Butler and B. Epel, Biochim. Biophys. Acta 205 (1970) 507.
[5] M.D. Kamen, T. Horio, Ann. Rev. Biochem. 39 (1970) 673.
[6] K. Miki, J. Sekuzu and K. Okunuki, Ann. Rep. Biol. Works Fac. Sci. Osawa Univ. 15 (1967) 33.
[7] H. Yaoi and H. Tamiya, Proc. Imp. Acad. Tokyo 4 (1928) 436.
[8] B.B. Rubenstein, Science 74 (1931) 419.
[9] D.A. Webster and D.P. Hackett, Plant Physiol. 41 (1966) 599.
[10] H. Ninneman, manuscript submitted.