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LIGHT DEPENDENT OXYGEN UPTAKE BY ANACYSTIS NIDULANS, STUDIES WITH ENDOGENOUS AND ADDED REDUCTANTS*

J. W. KLEINEN HAMMANS**, T. TEERLINK and J. Th. VAN DEN BERG,

Vakgroep Biofysica, Laboratorium voor Experimentele Fysica, Utrecht

SUMMARY

The two pigment systems, with optimum activity at 675 nm and 750 nm, that are responsible for light dependent oxygen uptake by *Anacystis nidulans* remain active in the presence of DCMU. The activity of the 750 nm system is increased at the beginning of the phase of exponential growth when L-glutamate has been added to the growth medium. We presume that variations in growth conditions may induce the formation of endogenous reductants that cause increased oxygen uptake. Experiments with added reductants showed that L-ascorbate, $DCPIPH_2$ and TMPD are readily photooxidized in the intact cell by the 750 nm system.

Abbreviations: DCMU: 3-(3,4-Dichlorophenyl)-1,1-dimethyl urea. DCPIP: 2,6-Dichlorophenol-indophenol. EDTA: Ethylene diaminetetra-acetic acid. TMPD: N,N,N',N'-Tetramethyl-p-phenylenediamine.

1. INTRODUCTION

Photosynthetic reduction of oxygen is thought to be associated with photoreaction I and has been studied extensively in recent years (e.g. ELSTNER et al. 1976). VAN BAALEN (1965) described light dependent O_2 uptake by *Anacystis* nidulans with uric acid as added reductant. The most effective wavelength was found to be around 750 nm. He concluded that the sensitizing pigment was identical with or closely related to a pigment called P750, which had been isolated earlier by GASSNER (1962). KLEINEN HAMMANS et al. (1977) measured action spectra of light-induced oxygen uptake by *Anacystis* without inhibitors and artificial redox compounds. Two separate systems were found, one activated by 675 nm light, the other by 750 nm light.

In this report we discuss further experimental results which suggest that for both systems electrons are supplied by endogenous reductants.

We also present data showing that activity of the 750 nm system can be stimulated by the addition of certain artificial reductants.

* Dedicated to Professor Dr. J. B. Thomas, upon his retirement from the chair of Biophysics.

** Postal address: Physics Laboratory, State University, Sorbonnelaan 4, Utrecht, The Netherlands.

2. MATERIAL AND METHODS

Methods for growing P750 enriched *Anacystis* and recording of polarographic action spectra have been described elsewhere by KLEINEN HAMMANS et al. (1977).

Quantitative measurements of oxygen uptake were made with a Hansatech D. W. Clark-type O_2 electrode. Cells were precipitated by centrifugation and resuspended in 0.02 M Tris-HCl, pH 7.7. Assay mixtures consisted of 2.0 ml cell suspension (containing 40 μg chlorophyll) to which 5 μmoles of reductant were added from a stock solution. Appropriate volumes were taken from the following stock solutions: 20 mM L-ascorbic acid/Na ascorbate + 2 mM EDTA-Na salt, pH 6.6; 20 mM TMPD; 20 mM potassium ferrocyanide; and 10 mM $DCPIPH_2$, prepared by adding a small excess of 20 mM DCPIP solution to ascorbate stock solution. The reaction chamber of the electrode was illuminated with far red light ($\lambda > 710$ nm). The beam from a 150 W projector was passed through a water filter and a Kodak cut-off filter (transmittance 1% at 700 nm, 20% at 725 nm and 30% at 750 nm), both of which were fitted inside the projector. The light intensity on the sample, measured at 750 nm (interference filter $\lambda_{1/2} = 11$nm) was about 40 $W \cdot m^{-2}$.

Growth media with L-glutamate contained 2 mmoles of L-glutamic acid/l; pH was adjusted to 7.4 by the addition of solid Na_2CO_3.

3. RESULTS AND DISCUSSION

When illuminated, cells of *Anacystis* that have been incubated in 1 mM DCMU are no longer capable of oxygen production. The two systems responsible for photoreduction of oxygen are, however, still active (*fig. 1*), so it is unlikely that electrons are supplied via a photosystem II reaction. Metabolites from dark reactions may serve as endogenous electron donors. A similar suggestion has been made with regard to the light dependent oxygen uptake by *Anabaena variabilis* (MURAI & KATOH (1975)). WANG et al. (1977) suppose that in *Anacystis* part of the light energy absorbed by phycocyanin is conveyed to photoreaction I. No band of phycocyanin is seen in the spectrum of *fig. 1*, and the position and half-width of the 675 nm band indicate that the long wavelength forms of chlorophyll *a* do not participate in the 675 nm uptake system. It is questionable therefore whether one and the same aggregate of pigments is used both for photoreaction I and the 675 nm system.

L-glutamic acid, which is involved in carboxylic acid as well as nitrogen metabolism, was chosen to test whether formation of endogeneous reductants can be induced. *Anacystis* in a medium containing 2 mM L-glutamate per l grows very slowly during the first 24 hours after incubation. After this period the usual phase of exponential growth begins. A marked increase of 750 nm system activity is observed during the first hours of the exponential growth phase, even at 25 °C, a temperature at which normally no activity of the 750 nm system can be detected (*fig. 2*). The activity of the 750 nm system reverts to

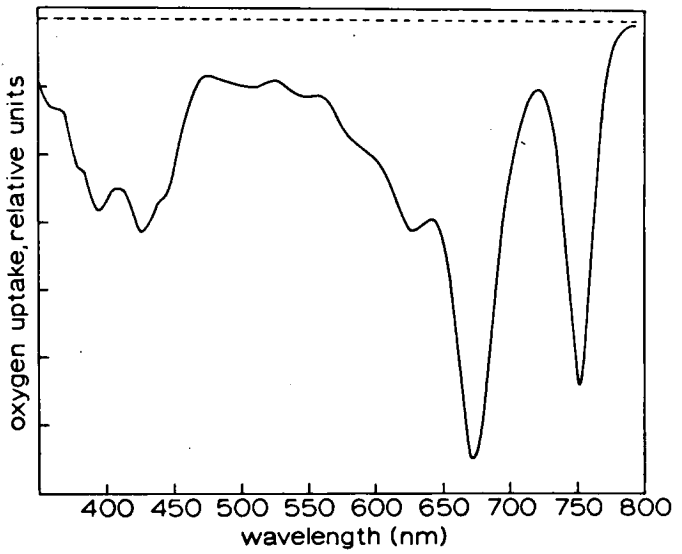

Fig. 1. Action spectrum of light dependent oxygen uptake by cells of *Anacystis* after incubation in 1 mM DCMU. The sample was kept at 40°C during recording of the spectrum.

normal proportions within one day of further growth.

Addition of artificial reductants to *Anacystis* may cause stimulation of the 750 nm system. Some results are listed in *table 1*. We did not observe any stimulation when ferrocyanide was added. As can be seen from *table 1*, we were also

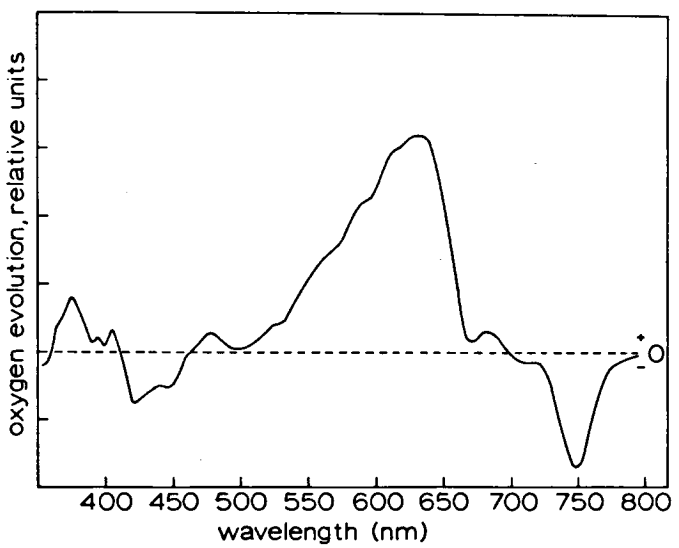

Fig. 2. Action spectrum (at 25°C) of cells grown in a medium containing L-glutamate.

Table 1. Oxygen uptake by *Anacystis* cells irradiated with far red light. For details see "Material and Methods".

Reductant	E_0 at pH 7 (DAWSON *et al.* (1969))	O_2 consumed (μmoles·hour^{-1}·μg chlorophyll^{-1})
Ascorbate	+0.058 V	3.68
$DCPIPH_2$	+0.217 V	0.87
TMPD	+0.260 V	0.71
$Fe(CN)_6^{4-}$	+0.360 V	0.00
none	–	0.00

unable to detect any oxygen uptake by untreated cells when they were illuminated with far red light. However, action spectra (e.g. *fig. 1*) clearly show that the 750 nm photoreaction does not require the addition of reductants. We conclude that the method used for the quantitative measurement of O_2 uptake is less sensitive than the modulated light technique used for recording the action spectra. The possibility that ferrocyanide or compounds with even more positive redox potentials may supply electrons for the 750 nm light reaction should not be ruled out completely.

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REFERENCES

DAWSON, R. M. C., D. C. ELLIOTT, W. H. ELLIOTT & K. M. JONES (1969): *Data for Biochemical Research*, Oxford.

ELSTNER, E. F., G. F. WILDNER & A. HEUPEL (1976): Oxygen Activation by Isolated Chloroplasts from Euglena gracilis. Ferredoxin-dependent Function of a Fluorescent Compound and Photosynthetic Electron Transport close to Photosystem I. *Arch. Biochem. Biophys.* **173**: 623–630.

GASSNER, E. B. (1962): On the pigment absorbing at 750 mμ occurring in some blue-green algae. *Plant Physiol.* **173**: 623–630.

KLEINEN HAMMANS, J. W., G. H. HENDRIKS & T. TEERLINK (1977): Light dependent oxygen uptake by the blue-green alga Anacystis nidulans. *Biochem. Biophys. Res. Commun.* **74**: 1560–1565.

MURAI, T. & T. KATOH (1975): Photosystem I-dependent oxidation of organic acids in blue-green alga Anabaena variabilis. *Plant & Cell Physiol.* **16**: 789–797.

VAN BAALEN, C(1965): The photooxidation of uric acid by Anacystis nidulans. *Plant Physiol.* **40**: 368–371.

WANG, R. T., C. L. STEVENS & J. MYERS (1977): Action Spectra for Photoreactions I and II of Photosynthesis in the blue-green alga Anacystis nidulans. *Photochem. Photobiol.* **25**: 103–108.