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OXYGEN EVOLUTION FROM LYOPHILIZED ANACYSTIS WITH CARBON DIOXIDE AS OXIDANT

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SUMMARY

Lyophilized *Anacystis nidulans*, when resuspended in phosphate buffer (0.05 M phosphate, 0.1 M KCl, 0.04 M $MgSO_4$; pH 7) evolve O_2 when CO_2 is used as a hydrogen acceptor. Absorption and fluorescence characteristics of the lyophilized material and the effects of different intensity and wavelength of light on O_2 exchange, are reported.

INTRODUCTION

Cell-free systems of photosynthetic organisms, which retain certain biological functions of the intact cells, are especially advantageous for biochemical and spectroscopical investigations, since they are more amenable to fractionation and chemical treatment. In the present work, we attempted to assess the biochemical integrity of a freeze-dried (lyophilized) preparation of *Anacystics nidulans*, as far as photosynthesis is concerned. It has been shown by GERHARDT AND TREBST[1] that such a system, prepared from a suspension of blue-green algae in 5 % sucrose–Tris buffer (pH 7), has a good capacity for O_2 evolution in light with Hill oxidants (quinones, ferricyanide, $NADP^+$) and for cyclic and non-cyclic ADP-phosphorylation. (For earlier work see ref. 1.) In the present paper, experiments are described which show that the *Anacystis nidulans* lyophilizate has photosynthetic capacity, and is able to evolve O_2 in light also with CO_2 as oxidant.

MATERIALS AND METHODS

Anacystis nidulans, grown under low-light conditions[2], was lyophilized according to the procedure described by GERHARDT AND TREBST[1]. Cells were washed once with distilled water and then suspended in 5 % sucrose solution in 0.02 M Tris buffer (pH 7.6). The suspension was cooled to −60° and freeze-dried (lyophilized) overnight at −60° under low (0.5 mm of Hg) pressure. The dry powder was stored over P_2O_5 at 0°.

For O_2 exchange studies, the platinum-electrode polarograph, described by BANNISTER AND VROOMAN[3] was used. A paste of cell material was prepared by resuspending the lyophilizate in a buffer, consisting of 0.05 M phosphate, 0.01 M

* Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

KCl, 0.04 M $MgSO_4$ at pH 7, and this was spread on the platinum electrode of the polarograph. The same buffer served as conducting medium. During the polarographic studies, air containing 5 % CO_2 was bubbled through the system at a steady rate. A negative potential of −0.4 V was applied to the platinum electrode and the current changes were amplified by a Keithley microammeter (model 150A) and recorded on a Varian G-10 recorder. The response of the polarograph to light was checked in the absence of lyophilized cell material and found to be zero. This rules out any complication arising from changes in diffusion rates or from convection currents due to local heating of the electrode area.

The illumination was provided by a Bausch and Lomb monochromator, with slits adjusted to 2 mm (bandwidth, 6.6 mμ). Light intensities were measured by an Eppley thermopile. (The energy output of the monochromator lamp at 600 mμ was 370 ergs·cm^{-2}·sec^{-1} at the platinum electrode.) The energies were converted into numbers of incident quanta and the recorder readings on the polarograph were reduced to equal number of incident quanta. For the measurements of the light curves of the photo-induced O_2 uptake, and of the initial O_2 spike, a high intensity tungsten lamp (3500 ergs·cm^{-2}·sec^{-1} at the platinum electrode) was used; the intensity was varied using Balzer's neutral density filters.

For absorption and fluorescence measurements, the lyophilized material was suspended in Warburg's carbonate–bicarbonate buffer No. 9. Absorption spectra were taken in a Bausch and Lomb spectrophotometer, fitted with an integrating sphere, using 1-cm cuvettes. The bandwidth of the measuring beam was 5 mμ. Fluorescence measurements were performed using an automatic spectrofluorimeter, described elsewhere[4], in which fluorescence is collected from the front surface. The light path of the exciting beam was 1.0 mm. Since dilute suspensions [$A_{680\ m\mu}$ (for 1.0 mm path) about 0.03] were used for these measurements, no corrections for reabsorption of fluorescence were applied. The emission spectra were corrected for the photomultiplier sensitivity; they were measured by inserting a Corning C.S. 2–63 sharp cut-off filter before the analyzing monochromator. Excitation spectra were normalized to equal numbers of incident quanta. Emission spectra were observed with a bandwidth of approx. 3 mμ; in measuring the excitation spectra, the exciting beam was 4 mμ wide.

All measurements were made at room temperature (22–25°).

RESULTS AND DISCUSSION

Absorption and fluorescence spectra of the suspension of lyophilized Anacystis

The absorption and fluorescence spectra of lyophilized Anacystis, suspended in carbonate–bicarbonate buffer No. 9, were measured in order to spectroscopically characterize our system. Fig. 1 shows the per cent absorption ($100(I_0 - I)/I_0$) spectrum (Curve A) and fluorescence excitation spectra (measured at 685 mμ (Curve B)) and at 745 mμ (Curve C). The ratio of absorbance at 622 mμ (phycocyanin peak) to that at 670 mμ (chlorophyll peak) is approx. 1.0. However, the ratio of fluorescence intensities (measured at 745 mμ), excited by 622 mμ and by 670 mμ light, respectively, is approx. 3.3. The relative inefficiency of chlorophyll *a* absorption in producting chlorophyll *a* fluorescence, is more clearly seen in Curves D and E in Fig. 1, which is a plot of the relative quantum yield of fluorescence as function of exciting wave-

length. Here, a decline in the fluorescence yield appears beyond 642 mμ. These results are qualitatively similar to those of DUYSENS[5] and FRENCH AND YOUNG[6] obtained with normal algal suspensions. The emission spectra excited by 440 mμ and 580 mμ

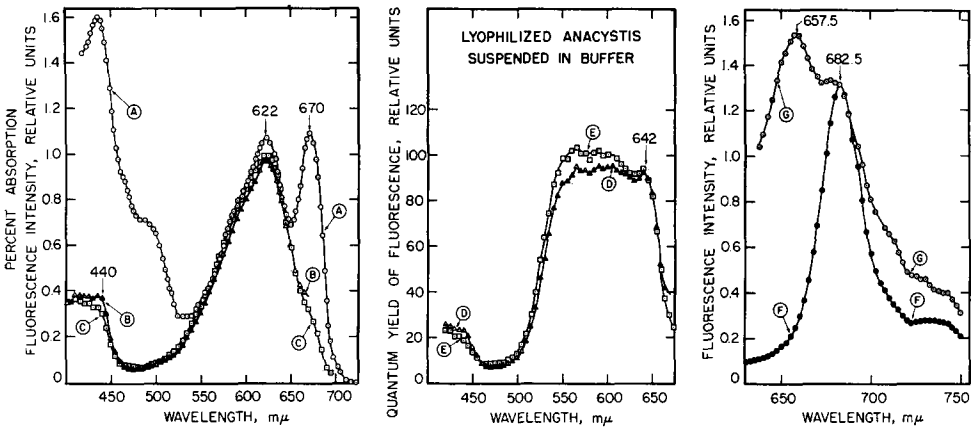

Fig. 1. Absorption and fluorescence spectra of lyophilized *Anacystis nidulans* suspended in carbonate–bicarbonate buffer. *Left*, A (○): per cent absorption spectrum $100(I_0 - I)/I_0$ or $(1 - 10^{-A}) \times 100$; B (△): action spectrum of chlorophyll *a* fluorescence (fluorescence intensity per incident quanta) measured at 685 mμ (cut-off filter C.S. 2–64) and C (□): the same measured at 745 mμ (cut-off filter C.S. 7–69). *Middle*, D (△): Quantum yield (fluorescence intensity per absorbed quantum) of chlorophyll *a* fluorescence measured at 685 mμ (with C.S. 2–64 before the analyzing monochromator) and E (□): quantum yield of chlorophyll *a* fluorescence measured at 745 mμ (with C.S. 7–69). *Right*: Emission spectrum (with C.S. 2–63 before the analyzing monochromator). F (●): excited by 440 mμ (with C.S. 4–72 on excitation light) and G (○): the same excited by 580 mμ. (The curves have been adjusted at appropriate wavelengths for better comparison.)

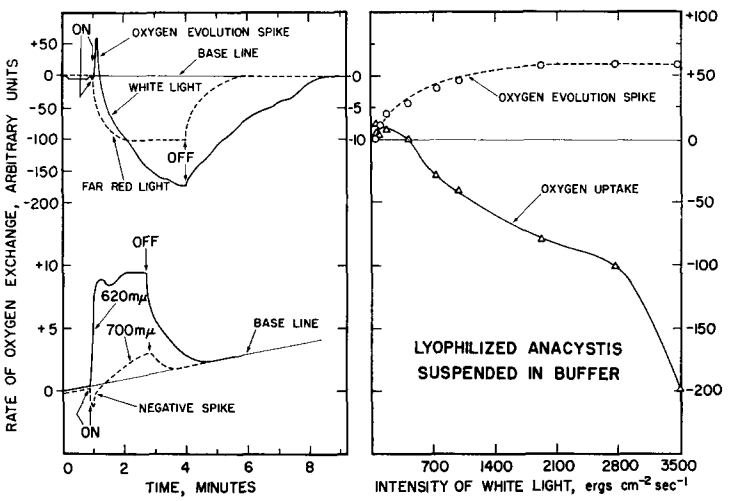

Fig. 2. *Left* (top): O_2 exchange in lyophilized *Anacystis nidulans* suspended in phosphate buffer with white light (———) and far-red light (– – – –) of high intensity. The scale for far-red light curve is given on the right side of the left figure. *Left* (bottom): O_2 exchange with 620 mμ (———) and 700 mμ (– – – –) light of low intensity. The time-course of O_2 exchange has not been corrrected for the intensity of exciting light. *Right*: Dependence of O_2 evolution spike and O_2 uptake on the intensity of illumination.

light are also shown in Fig. 1. Excitation by 440 mμ (Curve F) gives a single peak at 682.5 mμ, whereas fluorescence excited by 580 mμ has 2 peaks (Curve G): one at 658 mμ (phycocyanin) and one at 680 mμ (chlorophyll *a* fluorescence). These results also correspond to those obtained on whole cells[5–7].

The above results show that lyophilization did not disintegrate the pigment systems. In particular, we note that phycocyanin does not leach out.

Oxygen exchange with monochromatic light

Short-wave light. Short-wave light (620 mμ) clearly supports O_2 evolution with CO_2 as acceptor (solid curve, bottom left, Fig. 2). In monochromatic light (obtained from our set-up), the rate of O_2 evolution is proportional to the intensity of light. The following observations were made at these low-light intensities. Table I shows the rate of O_2 evolution (corrected for incident quanta) at several wavelengths. It is clear that light absorbed by phycocyanin (500–650 mμ) is more effective than light absorbed in chlorophyll *a* (400–480 mμ and 650–700 mμ) in O_2 production with CO_2 serving as an electron acceptor in the lyophilized Anacystis. These results with lyophilized material are similar to those of HAXO AND BLINKS[11] and BRODY AND EMERSON[12] on normal phycobilin-containing algae.

Far-red light. Far-red light supports O_2 uptake, as shown by the following qualitative observations: (i) when far-red light was removed from white light by

TABLE I

RATE OF O_2-EVOLUTION IN DIFFERENT WAVELENGTHS OF LIGHT FOR THE SAME NUMBER OF INCIDENT QUANTA, IN LYOPHILIZED ANACYSTIS (SUSPENDED IN PHOSPHATE BUFFER)

Wavelength (mμ)	*Rate (recorder units*)*	*Wavelength (mμ)*	*Rate (recorder units*)*
400	0	600	11
420	0	610	11
440	0	620	10
460	0	630	9
480	10	640	9
500	15	650	6
520	10	660	5
540	14	670	4
560	17	680	3
570	19	690	3
580	18	700	2
590	11	710	1
		720	0

* 100 recorder units = 0.1 μA.

appropriate filters, O_2 uptake was reduced; (ii) white light filtered through a Schott RG-8 filter gave high rate of O_2 uptake, but no O_2 evolution (Fig. 2, top left, dashed curve); (iii) long-wave monochromatic light (680 mμ, 690 mμ, 700 mμ, 710 mμ) shows a negative spike (O_2 uptake; Fig. 2, bottom left, dashed curve) as shown by FRENCH and co-workers[8] in normal whole cells. (See discussion of photooxidation by light in RABINOWITCH[9]; also see OWENS AND HOCH[10] for the effect of far-red light on the O_2 uptake in normal Anacystis cells.)

Oxygen exchange induced by white light

In normal whole cells, the rate of O_2 evolution increases progressively with increasing light intensity and finally attains saturation. In homogenates of whole cells, however, O_2 uptake is observed under high-light intensities and aerobic conditions. This photo-induced O_2 uptake increases with increasing light intensity and takes over O_2 evolution in bright light (unpublished observations). The lyophilized material used in the present work behaves like a homogenate (see below). Furthermore, we note that the white light used here contained a large proportion of far-red light—which was shown above to support O_2 uptake.

Under aerobic conditions, white light of high intensity (3300 ergs·cm^{-2}·sec^{-1}) obtained from an incandescent lamp gave an initial burst of O_2 evolution, followed by O_2 uptake (Fig. 2, top left, solid curve). Fig. 2 (right) shows that the rate of O_2 uptake increases steadily with increasing light intensity, while the O_2 evolution (the spike) increases linearly with light intensity and then saturates. At light intensities lower than 350 ergs·cm^{-2}·sec^{-1} no O_2 uptake is observed at all, while O_2 evolution is clearly seen. It must be pointed out that the platinum electrode measures the net O_2 exchange rates, *i.e.*, it does not monitor uptake and evolution separately. Because of this disadvantage, the real rates may be different than those described in the present paper.

CONCLUSIONS

That the whole photosynthetic apparatus of the lyophilized organism resuspended in buffer is functioning (although at a reduced rate) is apparent from the fact that the only oxidant present in our system was CO_2 and that DCMU was capable of stopping O_2 evolution (both in white and 620 mμ light; see Table II). The O_2 evolution (with monochromatic light) lasted almost a whole day. It decreased by about 50% in 4–6 h. When argon was flushed through the system, the rate of O_2

TABLE II

OXYGEN EXCHANGE IN LYOPHILIZED ANACYSTIS, ARBITRARY UNITS

Light	*Effect of DCMU: CO_2 as oxidant*			
	O_2 Evolution		*O_2 Uptake*	
	−DCMU	*+DCMU*	*−DCMU*	*+DCMU*
620 mμ	+18	0	0	0
White (including far-red)	+60	0	−250	−80

Light	*Effect of addition of $NADP^+$*		
	O_2 Evolution		
	(CO_2/Air)	*(Argon)*	*($NADP^+$/Argon)*
620 mμ	+18	+3	+32
White (including far-red)	—	—	+73

evolution was reduced by a factor of 6–10, confirming that CO_2 was used as the oxidant. The residual O_2 evolution with argon may be due to the use of some endogenous oxidants, or, more likely, to incomplete removal of CO_2. However, the lyophilized cells were much less efficient with CO_2 than with $NADP^+$ as oxidant; in the latter case, the O_2 evolution rate was twice as large—at least in one experiment (Table II). Preliminary estimates suggest that the O_2 evolution in our lyophilized cells is less than 10 % of that of normal *Anacystis nidulans* cells.

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