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MAXIMUM QUANTUM YIELD AND ACTION SPECTRUM OF PHOTOSYNTHESIS AND FLUORESCENCE IN CHLORELLA

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SUMMARY

The maximum yield of oxygen production in Chlorella was found, in new, systematic experiments, not to exceed 0.12, under a great variety of conditions. Measurements were made on autospores and other synchronous cultures, at different CO_2 concentrations, at very low light intensities, and in the presence of 'catalytic' amounts of blue light. The action spectra (quantum yield as a function of wavelength) of photosynthesis, and of chlorophyll *a* fluorescence *in vivo*, were measured on the same Chlorella cell suspensions. In both cases, a decline in the yield was observed in the red beginning at about 675–680 nm. The yield declines to 50 % of the maximum, on both curves, at 690–695 nm. A strong dip was observed in the action spectra of both fluorescence and photosynthesis at 660–665 nm.

INTRODUCTION

In the now widely accepted two-step model of photosynthesis[1,2], a minimum of 8 quanta are required to evolve one oxygen molecule. In the past, values less than 8 have been occasionally noted[3–5], even by observers who found the average requirement to be >8. If such low quantum requirements could be obtained consistently under reproducible conditions, the present model would have to be replaced. To settle this point as definitely as possible, we measured the quantum yield in Chlorella cultures grown under a variety of experimental conditions. Previous measurements in this laboratory[3] had been made on cultures grown in continuous light for 3 or 4 days, *i.e.*, on populations containing cells of all ages (meaning by 'age', time since the last cell division). It was suspected that the quantum yield may be larger in populations containing young cells only. We therefore grew algal cultures synchronously, and measured the quantum yield at various stages. As described in the section RESULTS AND DISCUSSION, no culture gave yields higher than 0.12.

The 'red drop' in the action spectrum of oxygen evolution had been known since the investigations of EMERSON AND LEWIS[6] in 1943, resumed by EMERSON, CHALMERS AND CEDERSTRAND[7] in 1957 (for review, see ref. 8). The drop of the quantum yield of photosynthesis in Chlorella (suspended in carbonate buffer No. 9) was found to begin, at room temperature, at 680 nm. A similar red drop in chlorophyll *a* fluorescence yield *in vivo* was discovered by DUYSENS[9] in 1952 at 675 nm. In our labora-

tory, SZALAY *et al.*[10], DAS AND GOVINDJEE[11], PAPAGEORGIOU[12] and WILLIAMS, MURTY AND RABINOWITCH[13] confirmed that the fluorescence drop begins at 675–680 nm; but it has been argued, particularly by WILLIAMS (unpublished), that the red drop of photosynthesis should start later—when pigment system I absorbs more light than system II—than that of fluorescence, which should begin when absorption by (weakly fluorescent) chlorophyll *a* in photosystem I increases relatively to that by strongly fluorescent chlorophyll *a* in photosystem II. The available data for comparing the two drops were unreliable, since they originated from experiments on different cultures, made under different conditions. (It is known[6,7] that the red drop in photosynthesis is found at slightly different locations under different experimental conditions.) We measured both drops in the same sample, at the same temperature, and in the same medium. The beginning of the drop is difficult to determine precisely, particularly in the measurement of O_2 evolution; a more reliable determination can be made of the wavelength at which the quantum yield drops to half its maximum value ('half drop'). As described in the section RESULTS AND DISCUSSION, the location of the half drop was found to be the same in the action spectra of photosynthesis and chlorophyll *a* fluorescence.

MATERIALS AND METHODS

Culturing of algae

Chlorella pyrenoidosa, Emerson's strain 3, was grown in nitrate medium (*cf.* ref. 14). Cells were harvested after 4 days, centrifuged, washed and resuspended in Warburg's carbonate buffer No. 9 for manometric and fluorescence experiments, and in phosphate buffer (pH 7.0) for polarographic measurements.

Synchronous cultures were grown at 30°, by a method similar to that of PIRSON, LORENZEN AND RUPPEL[15], using a regime of 14 h light and 10 h dark. Algae were harvested at different times in order to get cells in different physiological stages of development.

Absolute quantum measurements were made with differential manometers[3], by determining the pressure every minute with the help of cathetometers. Single measurements were also made at 20° and 10°. Light beams of 5–10 nm bandwidth were obtained from the Emerson–Lewis monochromator[6]. In the determination of action spectra, quantum yields of O_2 evolution were measured (at 5-nm intervals) as a function of wavelength.

Energy measurements were made with a large-surface bolometer[6]. A Bausch and Lomb spectronic 505 spectrophotometer, equipped with an integrating sphere, was used for absorption measurements. The readings obtained with this instrument agreed very closely with those obtained with the integrating spectrophotometer of CEDERSTRAND[16].

Comparative polarography

Warburg suggested that 'catalytic' amounts of blue light[17] and high CO_2 concentrations[18], are necessary to obtain a high quantum yield of photosynthesis (up to 0.37). We studied the effect of these conditions by comparative polarographic measurements. A platinum electrode, described by BANNISTER AND VROOMAN[19], was used, with a polarizing potential of −0.55 V. Algae were suspended in phosphate buffer, at pH 7; different CO_2–air mixtures were bubbled through the electrolyte; light

beams (half-bandwidth, 6.6 nm) were obtained from a large Bausch and Lomb monochromator.

Fluorescence measurements

Action spectra of fluorescence were measured for emission at 745 nm (*i.e.*, in the first vibrational satellite of the main F685 band), in a spectrofluorimeter[20,21]. The half-bandwidth was 13.2 nm for the measuring beam and 6.6 nm for the exciting beam. A Corning C.S. 7-69 red cut-off filter was placed at the entrance slit of the measuring monochromator. The observed fluorescence intensities were reduced to equal numbers of absorbed quanta to obtain relative quantum yields (Φ) as function of excitation wavelength (λ). (At the low light intensities used, fluorescence intensity was proportional to the rate of absorption.)

RESULTS AND DISCUSSION

We are interested in the maximum quantum yield of photosynthesis, and thus we should work in the low light intensity range and not under saturating light intensities. We made sure that this was the case in all the experiments reported below.

Effect of blue light

Table I indicates no stimulation of the rate of oxygen evolution, caused by far-red light (700–720 nm) or red light (650 nm), by addition of 'catalytic' amounts of blue light (480 or 490 nm), as reported by WARBURG, KRIPPAHL AND SCHROEDER[17]. We observed an enhancement only when strong 480-nm light was added to far-red light—the well-known EMERSON effect[7,8,22,25].

Effect of variations in CO_2 concentration

Fig. 1 shows the light curves of O_2 evolution at different concentrations of CO_2 in air. At the low light intensities used, the yield is the same whether 0.5, 5, or 10 %.

TABLE I

RATE OF O_2 EVOLUTION

In relative units.

Far-red light alone (700–720 nm)	*Blue light alone (480 nm)*	*Far-red + blue*	*Red light alone (640 nm)*	*Blue light alone (490 nm)*	*Both lights together*
30	3	33	50	4	54
			50	2	52

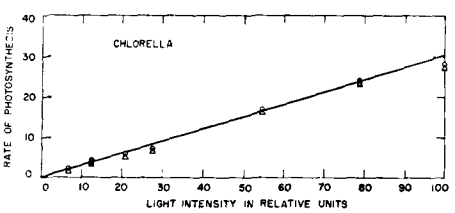

Fig. 1. Light curves of photosynthesis (in the low intensity range) at three different CO_2 concentrations. △, 0.5 %; ○, 5 %; ●, 10 %.

CO_2 are present in the air bubbled through the electrolyte. This observation, too, contradicts the findings of WARBURG[18].

Maximum quantum yield. Synchronous cultures

Table II shows the maximum quantum yield of photosynthesis at 680 nm, measured at about 0.4 μEinstein absorbed per sec per cm^2, at different stages of development. Experiments were made at 10°. The quantum yield remained the same (about 1/8) up to 6–8 h after the beginning of illumination, *i.e.*, it did not depend on the 'age' of the culture. However, the quantum yield declined after 8 h and was down to 0.07 after 1 h in the dark following 14 h exposure to light.

Similar results were obtained recently by SENGER AND BISHOP[23], who used synchronous Scenedesmus cultures. However, the maximum quantum yield obtained in their experiments was only about 0.09, as compared to 0.12 in our experiments.

Action spectra of photosynthesis and fluorescence

For these experiments, Chlorella cells were grown for 3 days over a 40-W incandescent bulb and one 15-W fluorescent ring, and for the last day over one 40-W incandescent lamp only. That cells grown in this way had low respiration and high quantum yields of photosynthesis were noted by EMERSON AND LEWIS[24]. Photosynthesis and fluorescence measurements were made on one and the same sample.

TABLE II

MAXIMUM QUANTUM YIELD OF PHOTOSYNTHESIS AT 680 nm

Conditions	*Quantum yield (at 680 nm)*
Young cells (autospores) taken 2 h after the beginning of the light stage of the growth cycle	0.12
Young cells, taken after 6 h exposure to light	0.11
Mature cells, taken after 14 h exposure to light	0.08
Mature cells, taken after 14 h of exposure to light, and 1 h in darkness	0.07

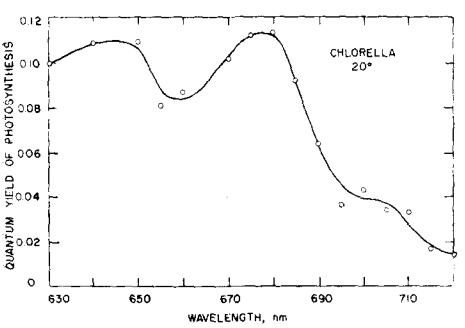

Fig. 2. Quantum yield of photosynthesis as a function of wavelength in Chlorella.

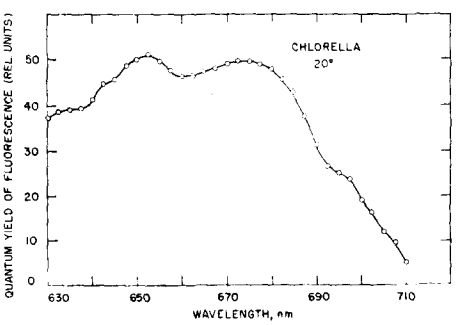

Fig. 3. Relative quantum yield of chlorophyll *a* fluorescence as a function of wavelength in Chlorella.

The action spectrum of photosynthesis (Fig. 2) shows a strong dip around 660 nm; the terminal red drop begins at about 680 nm. The action spectrum of fluorescence (Fig. 3) also shows a dip at 660 nm; the red drop begins at about 675 nm. The wavelength at which the yield drops to 50% of the maximum, lies, on both curves, at 690–695 nm. An inflexion of the fluorescence action curve at about 695 nm was observed in several experiments and thus seems reproducible; in the action spectrum of photosynthesis, measurements in this range also show a similar inflection at 700 nm, but these points are not as precise as those obtained in fluorescence measurements. The decline in the fluorescence action spectrum below 650 nm, observed also by PAPAGEORGIOU[12], is also reproducible; in this case, too, the photosynthesis curve was not precise enough to prove the occurrence of a similar decline.

The dip in the action spectrum of photosynthesis in the neighborhood of 660 nm, was first observed by EMERSON AND LEWIS[6]. MYERS[25] suggested that this may be due to absorption in chlorophyll *b* (which is more abundant in system II!). However, the dip is located at 660 nm and not at 650 nm, where the chlorophyll *b* absorption reaches its peak. A similar dip was observed by GOVINDJEE AND BAZZAZ[26] in the action spectrum of $K_3Fe(CN)_6$ reduction by spinach chloroplasts, and by PAPAGEORGIOU[12] and LITVIN AND SINESHCHOKOV[27], in the action spectra of chlorophyll *a* fluorescence in Chlorella and Elodea, respectively. In the present study, we have consistently observed a dip of up to 20% in the action spectra of both photosynthesis and fluorescence (Figs. 2 and 3). Perhaps this dip is caused by a short-wave component of a double absorption band belonging to a dimeric, (otherwise aggregated) form of chlorophyll *a*, rather than by excess absorption in chlorophyll *b* in system II; the long-wave component of this doublet can be supposed to account for the red drop above 680 nm.

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