Photosynthetic Adaptation in *Synechococcus* Cells

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Z. Naturforsch. **37 c**, 1075–1080 (1982); received August 12, 1982

Pigmentation, Quantum Requirement, Absorption Spectra, Oxygen Evolution, P 700, *Synechococcus*

The cyanobacterium *Synechococcus* (*Anacystis nidulans*, strain L 1401-1) grown under different light conditions showed variations in pigmentation. Ratios of photosynthetic pigments and the effect on quantum requirement and oxygen evolution were studied. An increase in the ratio of chlorophyll a forms with absorption maxima in the far red regime to total chlorophyll a forms was observed in cells grown in strong white light. The quantum efficiency of orange light (637 nm) – absorbed by phycocyanin – was higher after growth of *Synechococcus* in white than in red light. The quantum efficiency at 677 nm increased when cells were grown in red light and decreased strongly after transfering red light grown cells to conditions of strong white light. The results show an adaptation of pigment composition to light regimes during growth and its effect on photosynthesis.

In several unicellular cyanobacteria phycocyanin is the main light-harvesting pigment and sequestered in phycobilisomes. Pigmentation of cyanobacteria – *Synechococcus (= Anacystis nidulans)* was mainly used – can be changed by variation of conditions during growth *e.g.* light intensity, wavelengths [1 – 4] and CO_2 concentration [5]. The influence of that different pigmentation on several metabolic processes was studied by several workers: Action spectra measurements of PS I and PS II [6, 7] as well as investigations of absorption and fluorescence showed that in *Synechococcus* chlorophyll a is mainly active in PS I. The proportion of the two reaction centers can markedly vary in dependence on the conditions during growth [9]. The effect of different phycocyanin chlorophyll a ratios on energy transfer from phycocyanin to chlorophyll a were studied by several workers [10 – 12]. Investigations with phycocyanin-rich and phycocyanin-deficient cells showed that a direct energy transfer between phycocyanin and chlorophyll a may exist in *Synechococcus* [13 – 15].

We have reported previously the effect of light intensity and light quality on ultrastructure, lipid and fatty acid composition of *Synechococcus* [16, 17]. In cells grown in high white light a significant decrease of phycobilisomes and a slight distortion of thylakoid membranes was found. Therefore we have studied absorption spectra, quantum efficiency and action spectra of differently pigmented *Synechococcus* cells to obtain information on the influence of growth conditions on energy transfer and photosynthetic adaptation.

Materials and Methods

Synechococcus (= *Anacystis nidulans*, strain L-1402-1) obtained from the Algenreinkulturensammlung, Göttingen was grown at red light ($20\times10^3\,\mu W\cdot cm^{-2}$; > 650 nm) and white light of two different intensities (0.6 and $30.8\times10^3\,\mu W\cdot cm^{-2}$) under normal air conditions (0.03 vol.% CO_2). For more details see Döhler and Datz [17].

Absorption spectra of these *Synechococcus* cells were measured at room temperature (+ 20 °C) and at low temperature (– 196 °C; liquid nitrogen) with a special apparatus described by Leclerc *et al.* [18]. The absorbance at 680 nm of the different samples varied from 0.6 to 1.0. For more details and for calculation of difference spectra see [18]. The ratio of P 700/total chlorophyll a was estimated at – 196 °C by difference spectrophotometry with a dark refer-

Abbreviations: PS I, photosystem I; PS II, photosystem II; *A*, absorbance; HWL, high white light.

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ence stabilized with 2 mM ascorbate and by a flashed sample [19]. The P 700/chl ratio was calculated in absolute values by

$$\frac{A_{704} + \frac{A_{692} + A_{735}}{2}}{A_{678}}$$

and represents the molar ratio multiplied by an unknown constant. The molar extinction coefficient of the different chl a forms *in vivo* are not precisely known. Chlorophyll a and phycocyanin were estimated according to the method of Myers and Kratz [20].

Action spectra of oxygen evolution of *Synechococcus* suspensions were measured under constant light at + 25 °C with a Clarke oxygen electrode in the laboratory of Orsay, France. The monochromatic light was provided with a 1.8 nm band width of wavelengths corresponding to the main absorption bands of phycocyanin and chlorophyll a forms. The intensity of light was 2 to 3 nE · s^{-1} · cm^{-2} and the chlorophyll a content of the samples about 11.8 µg/ml. The oxygen concentration data obtained in 10 s rhythm were used for regression calculations with a micro computer (Hewlett-Packard 9810 A) with a precision of 10^{-4}. Results of photosynthetic oxygen evolution were corrected by the O_2 absorption during a 3 min dark period after a preillumination period of 3 min.

Quantum requirement measurements of the differently pigmented *Synechococcus* cells were carried out with a silicon solar cells device correcting a large part of the light scattering (arrangement described in more details by Leclerc *et al.* [21]). The device was calibrated at the different wavelengths with a bolometer of H. Röhrig.

Results

Figure 1 presents absorption spectra at − 196 °C of *Synechococcus* cells grown under various light conditions. Absorption spectrum of cells grown in strong white light (curve c) exhibits a significant shoulder in the far red region which can be attributed to enriched far red chlorophyll a forms. The amount of phycocyanin was strongly diminished; the absorption at 635 nm was due mainly to second-

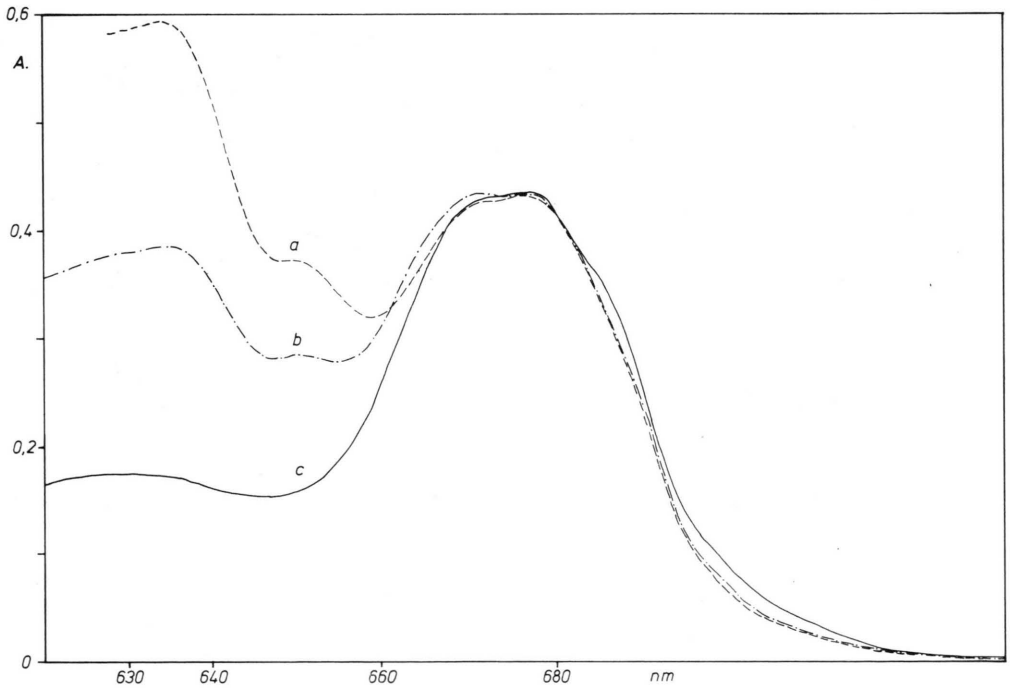

Fig. 1. Absorption spectra of *Synechococcus* cells at − 196 °C. Cyanobacteria were grown under different light conditions: a) in red light (> 650 nm; 20×10^3 µW · cm^{-2}), b) in low white light (0.6×10^3 µW · cm^{-2}) and c) in strong white light 30.8×10^3 µW · cm^{-2}). The spectra are adjusted at 680 nm; A = absorbance.

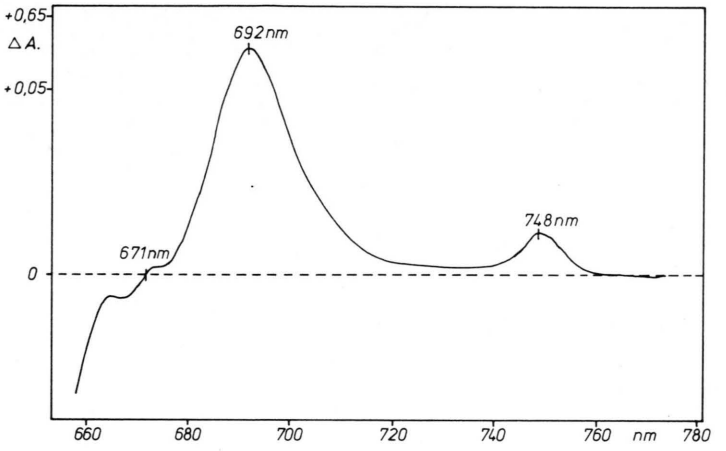

Fig. 2. Difference absorption spectrum of *Synechococcus* at − 196 °C. The spectrum was measured with strong white light grown cells as sample and red light grown cells as reference. The zero point is 671 nm and the absorbance of the reference 0.4 at 671 nm. *A* = absorbance.

ary vibrational bands of the chlorophyll a forms. Contrary to these cells in *Synechococcus* cultures grown under low white light (curve b) or under red light conditions (curve a) a decreased proportion of far red chlorophyll a forms was found. A significant higher phycocyanin content could be obtained under these conditions (see curve a + b at 635 nm) in comparison to the in high white light (HWL) grown cells. The difference spectrum of Fig. 2 indicate that the maximal variation of long wavelengths chlorophyll a forms in HWL grown cells was obtained at about 692 nm. The observed absorption band at 748 nm was mainly found in HWL grown cells. Murata *et al.* [22] have attributed this absorption band to carotenoids of the cytoplasma membrane. On the other hand, uptake of oxygen observed with photosynthetic lamella of *Anacystis* after illumination with light at 749 nm was explained by a P-750-mediated photooxidation reaction [23].

The difference spectra presented in Fig. 3 demonstrate that one flash (0.4 mJ) could saturate the apparent oxydation of P 700 at the used low tem-

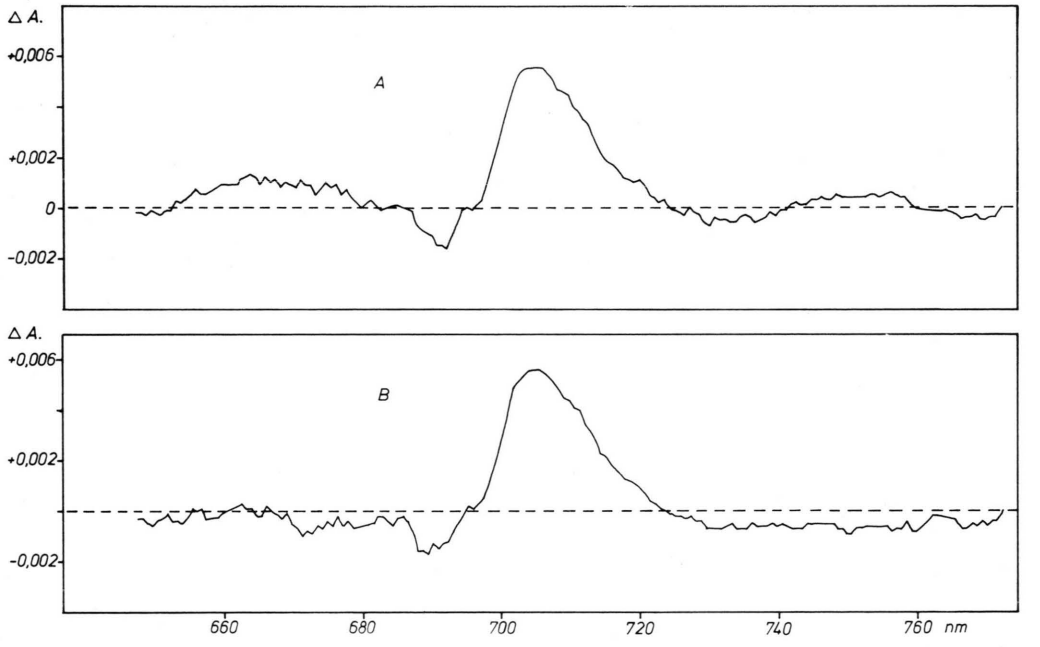

Fig. 3. Photooxidation of P 700 at − 196 °C with thylakoids of strong white light grown *Synechococcus* (0.7 absorbances at 680 nm) calculated by dark minus actinic light. A one flash (0.4 mJ), B three flashes in 5 s rhythm. *A* = absorbance.

perature (− 196 °C). No significant differences in the P 700/chlorophyll a ratios of *Synechococcus* cells grown under different light conditions were obtained. We found a variation between 0.006 and 0.01 estimated by the described method. Our results are in agreement with that of Kawamura *et al.* [9], who found also no variation of P 700 in low and strong white light grown cells.

Figure 4 shows action spectra of oxygen evolution and values of quantum requirement. Comparing the data of quantum requirement of cells grown under low (Fig. 4A) to that of strong white light grown cells (Fig. 4 B) no change could be observed at 677 nm. In HWL grown *Synechococcus* cells a significant enhanced efficiency at 700 nm was found. This corresponds to the higher proportion of the far red chlorophyll a forms. In HWL (part B) and in red light grown cells (part C) as decreased efficiency at 637 nm exists in comparison to cells grown under low white light; in this part of the spectrum phycocyanin is mainly absorbing. The observed decrease in the efficiency is independent on the phycocyanin content: the chlorophyll a/phycocyanin ratio decreased from 1 : 14 to 1 : 2. Therefore in this part of the spectrum the efficiency must be due to the vibrational secondary bands of chlorophyll a forms. In red light grown cells (Fig. 4 C) we found besides the decrease of the efficiency in light absorbed by phycocyanin a general increase of the chlorophyll efficiency. The best values could be measured at 661 nm, in a region of the absorption of chlorophyll a forms and allophycocyanin B.

Red light grown cells transfered to strong white light conditions (Fig. 4 D) showed after 3 days a

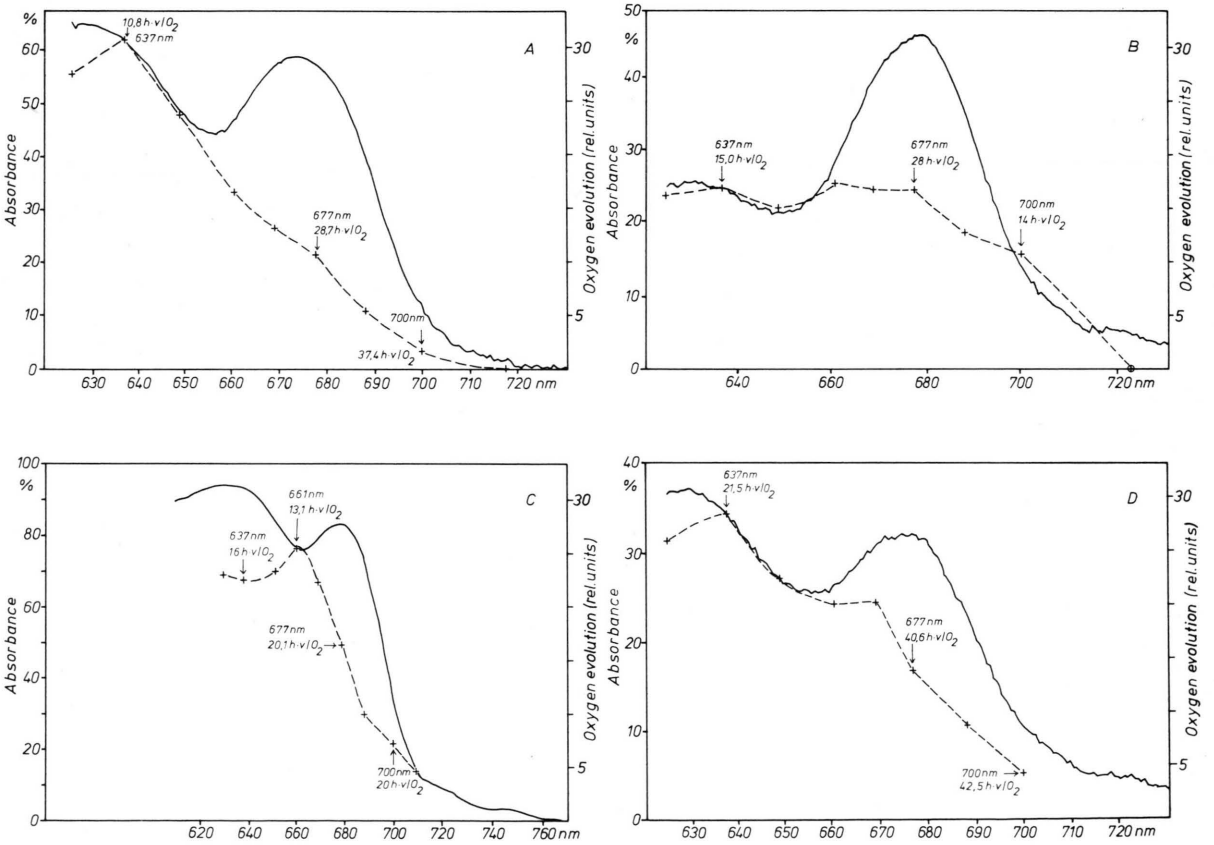

Fig. 4. Absorption and action spectra of oxygen evolution of *Synechococcus* grown under different conditions. Absorbance was measured directly in the reaction cuvette with actinic light different wavelength of 2 to 3 nE · $cm^{-2} \cdot s^{-1}$. The quantum requirements are shown at 637 nm, 677 nm and 700 nm of A) low white light grown, B) strong white light grown, C) red light grown and D) red light grown cells transfered to strong white light. In red light grown *Synechococcus* the quantum requirement at 661 nm is also presented. Absorbance of the suspension is given in % of incident light and the oxygen evolution in relative units.

small diminution of the phycocyanin/chlorophyll a ratio, only. This variation in light conditions during growth mainly resulted in a diminution of the quantum efficiency in the orange (637 nm) and red (677 nm) light part of the spectrum. Comparing the findings of in red light grown cells to these cells an increase of the quantum requirement from 16 h · v/O_2 to 21.5 h · v/O_2 at 637 nm and from 20.1 h · v/O_2 to 40.6 h · v/O_2 at 677 nm was found.

Discussion

Our results showed that the best quantum efficiencies were observed with *Synechococcus* cells grown under low white light conditions. These findings are in agreement with data of several other algae [24, 25]. Most efficient values were obtained with in low white light grown cells at 637 nm. In this part of the spectrum phycocyanin exhibits the absorption maximum. *Synechococcus* cells grown under high red or high white light conditions showed a sensibility of the efficiency at 637 nm: values of quantum requirement measurements varied from 10.8 h · v/O_2 to 15.0 or 16.0 h · v/O_2 (see Fig. 4) independent on the phycocyanin content of the cultures. The effect of strong light on *Synechococcus* – resulting in a decrease of photosynthetic yield in orange light (637 nm) – is more pronounced in cells grown in high red light followed by a HWL period (Fig. 4 D). In this part of the spectrum not only phycocyanin but also the secondary bands of chlorophyll a forms exhibit absorption maxima. This is mainly true for in strong white light grown cells. On the other hand, between 660 nm and 680 nm no significant changes in the quantum yield were found in cells grown under white light. These findings indicate that the organisation of chlorophyll a antenna must be not substantially changed by modification of light intensity.

Now the question arises: *why the effect of strong white light is more pronounced when the cells were previously grown in strong red light?* The further decrease of efficiency in the region of the absorption maximum of phycocyanin can be attributed to a partial destruction of the molecular arrangement of the phycocyanin caused by following strong white light period. Consequently the energy transfer must be partial inhibited within the phycobilisomes. The importance of the phycobilisome structure was shown by the better efficiency of allophycocyanin (Fig. 4 A, C and D; [26]). The observed strong effect on the chlorophyll efficiency (677 and 700 nm, see Fig. 4) can be only attributed to the light quality (red light) during the primary part of the growth period. The reason could be the labile organisation of the chlorophyll adapted to the directly absorbed light.

Why is the efficiency in the region of far red light enhanced when cells were grown under strong white light? The high quantum yield at 700 nm (14 h · v/O_2) of HWL grown *Synechococcus* cells can be attributed to the rather low amount of strong incident high white light which can be absorbed by the far red forms of chlorophyll a. This is indicative of a mechanism of photosynthetic adaptation to high incident energy. A part of the far red forms of chlorophyll a could act as a PS II antenna. Summarizing, our results can be interpreted by an adaptation of photosynthetic yield to strong light.

The relatively high oxygen evolution in far red light (700 nm) observed with HWL grown cells can be explained by a decreased respiratory oxygen consumption. Studies on oscillatory interaction of photosynthesis and respiration in *Anacystis nidulans* [27] support our interpretation.

Acknowledgements

We want to thank Prof. Dr. A. Moyse for his interest and Mrs. L. Tramp for the cultures control. Thanks are expressed to the "Deutscher Akademischer Austauschdienst", Bonn for the support of the visit of Prof. Dr. G. Döhler in France.

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