TRANSFER OF THE EXCITATION ENERGY IN *ANACYSTIS NIDULANS* GROWN TO OBTAIN DIFFERENT PIGMENT RATIOS

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ABSTRACT The blue-green alga, *Anacystis nidulans,* was grown in lights of different colors and intensities, and its absorption and fluorescence properties were studied. Strong orange light, absorbed mainly by phycocyanin, causes reduction in the ratio of phycocyanin to chlorophyll *a;* strong red light, absorbed mainly by chlorophyll, causes an increase in this ratio. This confirms the earlier findings of Brody and Emerson (12) on *Porphyridum,* and of Jones and Myers (8) on *Anacystis. Anacystis* cultures grown in light of low intensity show, upon excitation of phycocyanin, emission peaks at 600 mμ and 680 mμ, due to the fluorescence of phycocyanin and chlorophyll *a,* respectively. Changes in the efficiency of energy transfer from phycocyanin to chlorophyll *a* are revealed by changes in the ratios of these two bands. A decrease in efficiency of energy transfer from phycocyanin to chlorophyll *a* seems to occur whenever the ratio of chlorophyll *a* to phycocyanin deviates from the normal. Algae grown in light of high intensity show, upon excitation of phycocyanin, only a fluorescence band at 660 mμ and no band at 680 mμ. This suggests reduced efficiency of energy transfer from phycocyanin to the strongly fluorescent form of chlorophyll *a* (chlorophyll a_2) and perhaps increased transfer to the weakly fluorescent form of chlorophyll *a* (chlorophyll a_1).

INTRODUCTION

Algae containing phycobilins permit easier preferential excitation of one or the other of the two postulated photochemical pigment systems (1). The relative concentrations of chlorophyll *a* and phycobilins in algae depend on culture conditions. The photosynthetic efficiencies of cultures with different phycobilin-to-chlorophyll *a* ratios, have been studied in *Porphyridium cruentum* (2, 3) and in *Anacystis nidulans* (4). Brody and Brody (5) have studied induced changes in the efficiency of energy transfer from phycoerythrin to chlorophyll *a* in the red alga *Porphyridium.*

Here we present some observations on the fluorescence of *Anacystis nidulans* grown in lights of different color and intensity, and containing different proportions of phycocyanin and chlorophyll *a*.

EXPERIMENTAL PROCEDURES

Anacystis nidulans was obtained from the collection of Starr (6) (culture number 625). It was grown in an inorganic medium [medium C of Kratz and Myers (7)] to which an equal volume of Warburg's buffer #9 (15 parts of 0.1 M K_2CO_3 and 85 parts of 0.1 M $NaHCO_3$) was added to provide carbon dioxide.

Cultures were grown in white, orange, and red light. White light was obtained from a 750 watt tungsten lamp, orange light by filtering white light through a Balzers (Geraetebauanstalt, Balzers, Fürstentum Liechtenstein) K5 filter (a broad band interference filter with maximum tranmission at 600 mμ and a half band width of 30 mμ), and red light through a Corning (Corning Glass Works, Corning, New York) color specification (C.S.) number 2-64 sharp cutoff filter (50% transmission at 670 mμ and 85% transmission at wavelengths above 720 mμ). These light beams were called "high white," "high orange," and "high red," respectively.

Light intensities were measured by a Bi/Ag surface type Eppley thermopile (see Table I). The intensity of white light (high white) without any filters, was 2.0×10^7

TABLE I

LIGHT ENERGIES IN ERGS CM^{-2} SEC^{-1} USED FOR GROWING DIFFERENT CULTURES

"High white" light	2.0×10^7
"Low white" light	0.2×10^7
"High red" light	1.9×10^6
"Low red" light	0.2×10^6
"High orange" light	5×10^5
"Low orange" light	0.4×10^5

ergs cm^{-2} sec^{-1}. The low intensity beams ("low white," "low orange," and "low red") were obtained by using a 10% neutral density filter (Table I).

In general, cultures grown in light of high intensity, when compared with those grown in light of low intensity, showed larger vacuoles, fewer cells, and had a 10 times slower growth rate; they showed less dark material in electron micrographs and contained about ⅔ less pigment. (The electron micrographs were made by Shimony [unpublished].) The rate of photosynthesis (measured by manometry) of cells grown in white light of high intensity was five to ten times lower than that of "low light cells;" whereas the respiration rate of the "high light cells" was about 2 times higher.

The absorption spectra were measured in a Bausch and Lomb Incorporated (Rochester, New York) spectrophotometer (Spectronic 505) equipped with an integrated sphere, in a 1 cm deep aminco cuvette. The pigment ratios are characterized by comparison of the optical densities at 625 mμ (phycocyanin) and 678 mμ (chlorophyll *a*). Since chlorophyll *a* has some absorption at 625 mμ, and phycocyanin some absorption at 678 mμ, the optical density due to phycocyanin at 625 mμ and that due to chlorophyll *a* at

675 mμ, where calculated from the equations of Jones and Myers (8): (OD625) total = (OD625)PC + 0.244 (OD678) Chl, and (OD678) total = 0.110 (OD625)PC + (OD678) Chl, were OD stands for optical density, PC for concentration of phycocyanin, and Chl for that of chlorophyll *a*.

The fluorescence instrument was described before (9, 10). Fluorescence was observed from the same side on which the exciting light fell. The depth of the suspension was 0.25 cm. Emission spectra were corrected for photomultiplier sensitivity and for variations in the transmission efficiency of the analyzing monochromator, and the spectra were plotted in terms of relative numbers of quanta. Thin suspensions of algae were used to reduce the reabsorption of fluorescence. The band width of all slits in the monochromator was 6.6 mμ.

The degree of polarization of fluorescence was measured in some cultures with Weber's polarization instrument (11). The exciting light was mercury-cadmium light filtered through Corning glass filter, C.S. 4-72; the analyzing photomultipliers [EMI 9558B] were shielded by Schott (Jenaer glaswerk Schott & Gen., Mainz, West Germany) glass filters, RG-8. (The results of this experiment are mentioned only under the Discussion section.)

All experiments were made at room temperature (22°-25°C).

RESULTS

Absorption Spectra. The absorption spectra of *Anacystis* cells grown in white light of low intensity (labeled low white), in white light of high intensity (labeled high white), in orange light of low intensity (low orange), orange light of high intensity (high orange), red light of low intensity (low red), and in red light of high intensity (high red) are shown in Fig. 1. (All curves were adjusted to give the same value at 690 mμ.)

It is clear from these curves that the pigmentation of *Anacystis* depends strongly on the intensity and color of light used in growing the culture. Generally, the proportion of the pigment which best absorbs the light supplied during the growth period, is reduced when strong light is used–as if by photochemical decomposition [cf. Brody and Emerson (12) and Jones and Myers (8)]. The color of weak light does not affect significantly the proportion of pigments.

Florescence Spectra of Cells Grown in Red and White Light. The spectra of fluorescence excited by absorption at 580 mμ are presented in Fig. 2 (adjusted to coincide at 640 mμ), for cells grown in red light and white light of 2 different intensities. The fluorescence spectra have 2 major peaks: one at 660 mμ, due to phycocyanin, the other at 680 mμ, due to chlorophyll *a*. The latter is excited mainly by energy transfer from phycocyanin to chlorophyll [see Duysens (13) and French and Young (14)]. The differences between emission spectra shown in Fig. 2 can be explained by changes in the efficiency of energy transfer from phycocyanin to chlorophyll *a*.

The relative efficiencies of chlorophyll *a* fluorescence were calculated from observed fluorescence intensities and the estimated per cent absorptions. Table II shows the results. The ratio of absorbancies of phycocyanin (PC) and chlorophyll *a*

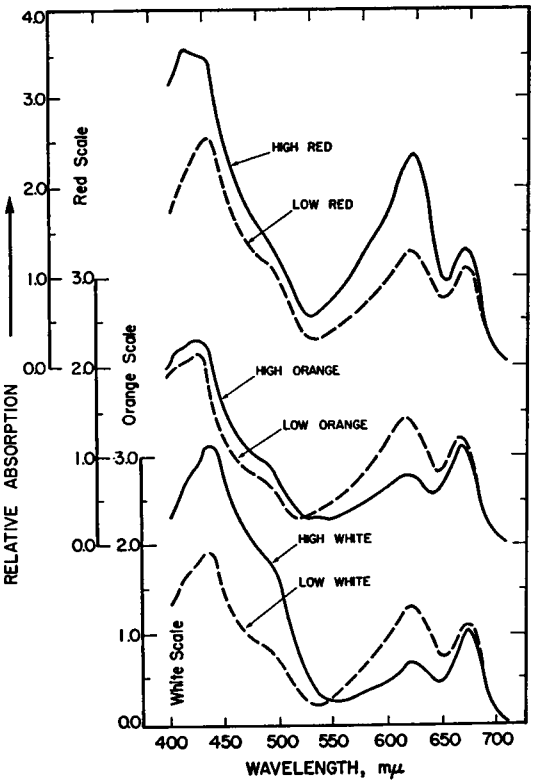

FIGURE 1 Absorption spectra of different cultures of *Anacystis nidulans* grown in lights of different intensity and color. Spectra of cells grown in high light intensities are shown with solid lines and those in low light intensities with broken lines. The ordinate labeled "white scale," "orange scale," and "red scale" are for cells grown in white, orange, and red light, respectively (see Table I and text).

(Chl *a*) were calculated by applying Jones and Myers (8) formulae (column 3). The relative efficiency of directly emitted chlorophyll *a* fluorescence (column 6) was calculated by dividing the fluorescence intensity at 685 mμ by per cent absorption at 430 mμ. (No energy transfer from carotenoids to chlorophyll *a* was assumed!) The fluorescence efficiency of phycocyanin fluorescence, upon excitation at 580 mμ (column 4) was obtained by dividing the fluorescence intensity at 660 mμ by the estimated per cent absorption of phycocyanin at 580 mμ. The fluorescence efficiency of phycocyanin-sensitized chlorophyll *a* fluorescence (column 5) was obtained from the relation: F685 − [f(Chl *a*) + f(PC)]/A(PC), where F685 = fluorescence intensity at 685 mμ; f(Chl *a*) = intensity of direct chlorophyll *a* fluorescence excited at 580 mμ (estimated from the efficiency of direct chlorophyll *a* fluorescence, as observed in the blue band, and the per cent absorption by chlorophyll *a* at 580 mμ); f(PC) = intensity of phycocyanin fluorescence at 685 mμ (estimated from the

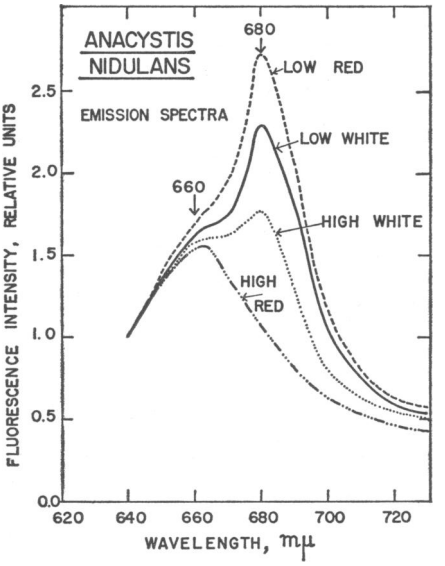

FIGURE 2 Florescence (emission) spectra of *Anacystis nidulans* (grown in red and white light); wavelength of exciting light, 580 mμ. The conditions of growth are indicated on the graph (see text for explanation). All emission spectra were corrected for variations in photomultiplier sensitivity and the efficiency of analyzing monochromator, in terms of quanta and not energy. Fluorescence intensities adjusted to coincide at 640 mμ are expressed in relative units.

TABLE II

CELLS GROWN IN RED AND WHITE LIGHT: FLUORESCENCE EFFICIENCIES (Φ), RELATIVE UNITS

			Excited at 580 mμ		Excited at 430 mμ
1	2	3	4	5	6
Sample no.	Culture conditions	Ratio PC/Chl a	Φ PC	Φ Chl	Φ Chl
1	"Low red"	1.00	1.89	2.86	0.67
2	"High red"	2.00	3.02	0.41	0.18
3	"High white"	0.74	2.46	1.45	0.61
4	"Low white"	1.05	2.21	2.06	0.40

fluorescence intensity of the same pigment at 660 mμ, and the ratio of fluorescence intensities at 685 mμ and 660 mμ in pure phycocyanin) (13); A(PC) is the estimated per cent absorption by phycocyanin at 580 mμ.

In red light cells, the chlorophyll *a* fluorescence efficiency, excited by 580 mμ, decreases from 2.86 to 0.41 when the phycocyanin fluorescence efficiency increases from 1.89 to 3.0 (columns 4 and 5, Table II). This suggests a decrease in transfer efficiency from phycocyanin to chlorophyll *a*. The same trend is seen in white light cells, when chlorophyll *a* fluorescence excited by 580 mμ decreases from 2.06 to 1.45, the phycocyanin efficiency increases from 2.21 to 2.46. In both cases, increase in phycocyanin fluorescence in "high light" cultures does not fully account

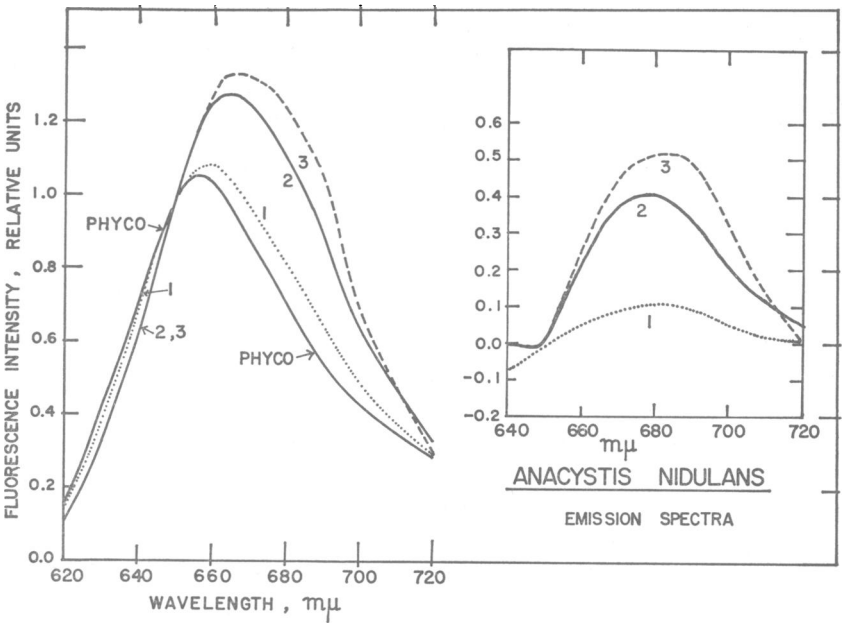

FIGURE 3 Fluorescence spectra of *Anacystis nidulans* grown in high intensity orange light; wavelength of excitation light, 580 mμ. Curves 1 through 3 show the spectra with samples grown in decreasing intensities (in 10% steps) of orange light. Insert shows the difference fluorescence spectra (ΔF) for chlorophyll *a* fluorescence [obtained by subtracting phycocyanin (Phyco) emission band]. Phycocyanin (Phyco) emission band is redrawn from Duysens (13).

for the decrease in chlorophyll *a* fluorescence; the latter is 2 to 3 times larger than the former. Since chlorophyll *a* in system I (Chl a_1) is supposed to be weakly fluorescent, these results may suggest increased energy transfer from phycocyanin to chlorophyll a_1, instead of chlorophyll a_2 in "high light cultures."

Transfer efficiency from phycocyanin to chlorophyll a_2 seems to decrease as the pigment ratio of phycocyanin: chlorophyll-*a* (PC/Chl) deviates from normal (which we assume to correspond to a ratio of about 1.0 in column 3).

Chlorophyll fluorescence efficiency in the red light cells and the white light cells, changes also when excited by light absorbed in chlorophyll *a* (column 6, Table II). This may indicate changes induced by the culture conditions in distribution of chlorophyll *a* between the two pigment systems (chlorophyll a_2 in system II is known to be more strongly fluorescent than Chl a_1 in system I). In these calculations (column 6, Table II), no corrections were made for the energy transfer from carotenoids to chlorophyll *a*; the differences may simply reflect changes in the latter.

Fluorescence of Orange Light Cells. The relative fluorescence yields in cultures grown and maintained in orange light of different intensities, show an almost constant chlorophyll *a* fluorescence efficiency, with a single peak at about

680 mμ when excited at 430 mμ light (absorbed mainly by chlorophyll *a*). In some samples, however, phycocyanin seems to absorb some light at 430 mμ (probably due to its high concentration), and a phycocyanin fluorescence peak is observed at 650 mμ.

The fluorescence emission spectra of orange light cells, upon excitation with 580 mμ light, are shown in Fig. 3. Curves 1 through 3 show the emission spectra for samples grown in orange light of decreasing intensity. These three samples were all grown in high intensity light but with intensity variations of about 10%. There is only a very small shoulder at about 680 mμ, indicating low efficiency of energy transfer from phycocyanin to chlorophyll *a;* the transfer efficiency increases as the intensity of light in which the cells were grown decreases. No quantitative conclusions were made from this set of experiments.

DISCUSSION

The intensity of the light in which the algae were grown is seen in this study to be of primary importance in determining the efficiency of energy transfer from phycocyanin to chlorophyll *a*. Both the color and the intensity of light determine the ratio of the concentration of the two pigments (see references 4, 8, and 12). Algae grown in red light of high intensity contain relatively more phycocyanin; cells grown in orange light of high intensity, relatively more chlorophyll *a*. Yet, the quantum yield of photosynthesis in both these samples is low, and energy transfer from phycocyanin to chlorophyll a_2, as indicated by sensitized chlorophyll *a* fluorescence (Fig. 2 and curve 1 in Fig. 3) is poor. The total pigment concentrations are low in all cells grown in high light. If local concentrations change (14) parallel to the total ones, this must make energy transfer less efficient.

Brody and Emerson (3) found that *Porphyridium* grown in the blue light had a lower quantum yield of photosynthesis (~0.05) when excited by green light (absorbed mainly in phycoerythrin) whereas those grown in green light had a higher quantum yield (0.09) under similar conditions of measurement. The authors suggested that this variability may be due to a change in the efficiency of transfer of excitation energy from phycoerythrin to chlorophyll *a*. Brody and Brody (5) later obtained evidence for changes in transfer efficiencies in *Porphyridium*. We have obtained similar evidence for changes in energy transfer in *Anacystis* cultures. Jones and Myers (4) earlier concluded that *Anacystis* grown in red light (BCJ red lamps) and having a lower chlorophyll *a* to phycocyanin ratio, show a lower activity of chlorophyll *a* in pigment system 1. (No conclusions concerning transfer efficiencies could be made in that work.) Since the growth conditions of Jones and Myers (4) and those used in the present paper were quite different, we do not attempt to correlate the two. (It would have been better if we had made comparable photosynthesis measurements on all our samples.)

In the two-pigment system hypothesis, chlorophyll *a* exists in two forms as

chlorophyll a_2 (chlorophyll *a* in system 2) and chlorophyll a_1 (chlorophyll *a* in system 1). Chlorophyll a_2 is often assumed to be (relatively) highly fluorescent, and chlorophyll a_1 weakly fluorescent (Duysens 15). The decreased efficiency of sensitized chlorophyll *a* fluorencence in algae grown under light of high intensity, must be due to a decrease in the transfer efficiency from phycocyanin to the "fluorescent" chlorophyll a_2. A decrease in transfer efficiency from phycocyanin to chlorophyll a_2 (in high light cells as compared with low light cells) may be accompanied by an increase in efficiency of transfer from phycocyanin to the "non-fluorescent" chlorophyll a_1, as evidenced by a larger decrease in chlorophyll a_2 fluorescence efficiency than accounted for by the increase in phycocyanin fluorescence efficiency. Furthermore, we have observed a slightly higher ratio of fluorescence intensity at 720 mμ to that at 682 mμ in "high intensity samples," as compared to the "low intensity samples." (No significant change in the ratio of fluorescence intensity at 692 mμ to that at 682 mμ was observed.) Since Vredenberg (16) (also see reference 10) has observed a shoulder around 730 mμ in the light-minus-dark difference spectrum, when cells of *Schizothrix calcicula* were excited with "system 1 light," our present observations may be taken to suggest an increased intensity of the chlorophyll a_1 band (730 mμ band?) in "high intensity cells." Furthermore, some experiments (made by us in Professor Weber's laboratory) show that *Anacystis* grown in orange light of high intensity has a 7% polarized fluorescence, as opposed to only 1% in cells grown in orange light of low intensity. Olson et al. (17) and Lavorel (18) have earlier ascribed polarized fluorescence to chlorophyll a_1. Altogether, it seems that in "high intensity cells," a lower efficiency of transfer from phycocyanin to chlorophyll a_2 is coupled with increased efficiency of transfer from phycocyanin to chlorophyll a_1.

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