J. Biosci., Vol. 2, Number 1, March 1980, pp. 63-68. © Printed in India.

Chromatic adaptation and photoreversal in blue-green alga *Calothrix clavata West*

A. S. AHLUWALIA, R. K. RAI and H. D. KUMAR
Department of Botany, Banaras Hindu University, Varanasi 221 005

MS received 23 August 1979; revised 19 November 1979

Abstract. Complementary chromatic adaptation, a well-established phenomenon in some blue-green algae, has been observed in *Calothrix clavata,* a heterocystous blue-green alga of the family Rivulariaceae. The chromatic adaptation has been observed for fluorescent and incandescent light by measuring the absorption spectra. The material grown in fluorescent light forms more of phycoerythrin whereas more of phycocyanin tends to be formed in incandescent light. Besides this, photoreversal was observed by transferring the incandescent light grown alga to fluorescent light conditions and vice-versa. Effect of photoreversal and chromatic adaptation has also been discussed for this alga under different monochromatic light conditions. The influence of different light conditions on morphological changes, heterocysts and hormogonia formation has also been investigated. Both chromatic adaptation and photomorphogentic phenolmena in this alga show the involvement of some photoreversible (red:green) pigment.

Keywords.. Chromatic adaptation; phycoerythrin; phycocyanin; *Calothrix.*

Introduction

Chromatic adaptation is one of the photomorphogenetic responses known to occur in blue-green algae. This is a phenomenon wherein the relative levels of different biliproteins are controlled by spectral quality of the light in which the algae are grown. These changes in pigment composition are usually due to differences in the relative rates of synthesis and total amounts of biliprotein pigments i.e. phycocyanin, phycoerythrin and allophycocyanin. Biliproteins are important accessory photosynthetic pigments and their ability to adapt chromatically confers an obvious ecological advantage in that the organism is able to make maximum photosynthetic use of the available light. This phenomenon has been well studied in a blue-green alga *Tolypothrix tenuis* (Fujita and Hattori, 1960a, b, 1962; Diakoff and Scheibe, 1973; Ohki and Fujita, 1978). Coupled with the changes at the subcellular level in pigment composition, gross macroscopic photomorphogenetic responses also occur (Bennett and Bogorad, 1973). In this work, we describe the red:green,incandescent: fluorescent light effects on biliprotein synthesis along with some photomorphogenetic changes in the blue-green alga *Calothrix clavata* West.

Materials and methods

Growth conditions

Calothrix clavata is a heterocystous blue-green alga. Its stock clonal axenic culture has been maintained on agar slants (1% v/v) at 28 ± 2°C, illuminated with 1500 lux of incandescent light, Allen and Arnon's medium (1955) has been used as the basal medium in all experiments.

Phosphate was autoclaved separately and added to cooled sterilised medium aseptically to avoid precipitation. In dark-grown culture experiments, 1% glucose (w/v) supported the growth of the alga.

Light treatments

The filaments grown in incadescent light were harvested,washed twice with sterilised double distilled water, and homogeneous suspension prepared by repeated shaking with sterilised glass beads. Equal aliquots from this suspension were inoculated in culture tubes and flasks and grown under different light conditions. Before giving short light exposures, the alga is kept in dark for 20 h to exhaust any carry-over effect of light. The intensity of both types of irradiance used i.e. incandescent and fluorescent, was 1500 lux. The filaments were grown in different light conditions for varying periods. After every exposure, 20 h dark incubation was given and then they were harvested for pigment analysis. Monochromatic red and green irradiance were produced by passing incandescent light through standard filters (Carolina Biological Supply Company, USA) and a water column to avoid any heating effect. Rest of the procedure is same as above.

Determination of absorption specturm

Pre-illuminated filaments were harvested by centrifugation (3000 rpm for 10 min) and washed with sterlised distilled water. After extraction of the pigments into 90% acetone, the water-soluble pigments, phycoerythrin and phycocyanin were extracted in distilled water by repeated freezing and thawing of the pellet. The absorbance spectrum was determined in a Spectronic-20 spectrocolorimeter in the wavelength region of 400-700 nm.

For photomorphogenetic studies, the filaments were grown on agar-plates with different light treatments as described above and the changes in their morphology, if any, were observed microscopically.

Results

The growth curves (figure 1) show that glucose (1%) in incandescent light conditions enhanced the growth of this alga. It also supported the growth in the dark but at a

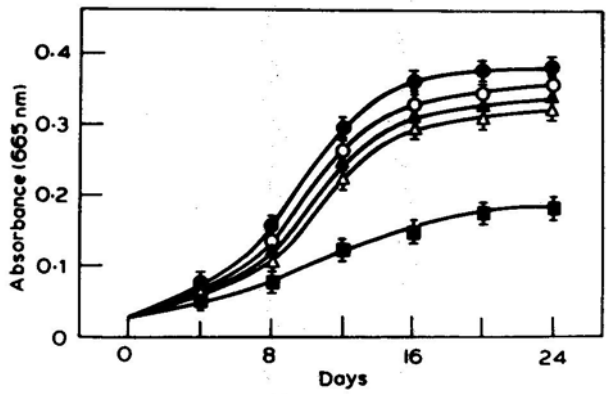
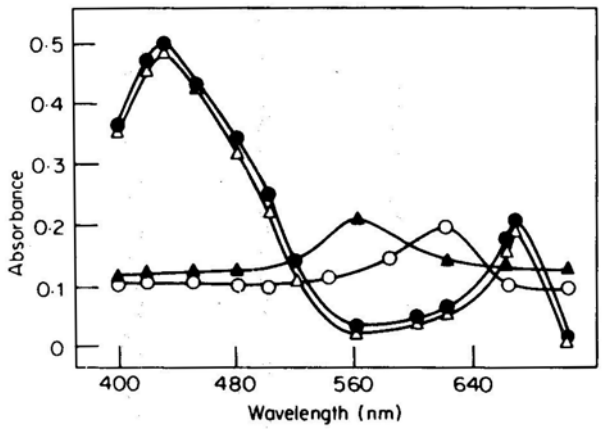


Figure 1. Growth of *Calothrix clavata under different conditions* 1 % glucose in dark; ■–■; Fluorescent light grown, Δ–.Δ; Incandescent light grown, ▲–▲; 20mM KNO_3 in incandescent light ●–●; 1 % glucose in incandescent light ○–○. 10 ml of cell suspension extracted in 10 ml of 90% acetone.

much reduced rate. Potassium nitrate (20 mM) slightly enhanced the growth. The growth in fluorescent light grown culture was approximately the same as that obtained in incandescent light.



Figured 2. Absorption spectra of acetone soluble pigments of fluorescent light grown culture Δ–Δ ; Incandescent light grown ●–● and water soluble pigments in fluorescent light grown ▲–▲; Incandescent light grown ○–○. Inoculum size same as in figure 1. On 10th day of growth, 10 ml of cell suspension extracted in 10 ml of extracting medium.

The absorption spectra for incandescent and fluorescent light grown cultures are shown in figure 2. The chlorophyll peaks were observed at 425 nm and 665 nm. The bili-proteins showed a characteristic pattern under different light conditions. There was only one peak at 560 nm in fluorescent light-grown culture and the peak shifted to 620 nm in the incandescent light grown cultures of same age and of nearly same chlorophyll content. The peak at 560 nm is due to phycoerythrin (purple pigment) and at 620 nm is due to phycocyanin (blue pigment). Similar results have been found by growing the cultures, supplied with glucose, under red and green lights. The culture could visually be distinguished on the basis of its colour (fluorescent light grown culture was brown in contrast to green under incandescent light conditions). The intermediate stage of reversal of pigment formation in this alga under different growth conditions in terms of light quality was determined (figure 3). When fluorescent light grown alga (9 days old) was transferred to incandescent light for three day, there was emergence of a small peak at 620 nm in addition to one larger peak at 560 nm (but still smaller than the control). Growth for additional 6-8 days in incandescent light spectrum clearly showed a sharp peak at 620 nm but no peak at 560 nm suggesting thereby the photoreversal of phycoerythrin to phycocyanin when light conditions shifted from fluorescent to incandescent (figure 3a). In figure 3b, the same phenomenon was observed but in the reverse direction i.e. when incandescent light grown culture was transferred to fluorescent light conditions, there was photoreversal of phycocyanin to phycoerythrin. When the incandescent light grown culture was transferred to fluorescent light conditions for three days, there was for mation of a small peak at 560 nm in addition to a peak at 620 nm. But after 6-8 days, there was only one peak 560 nm. The same pattern was obtained with red and green

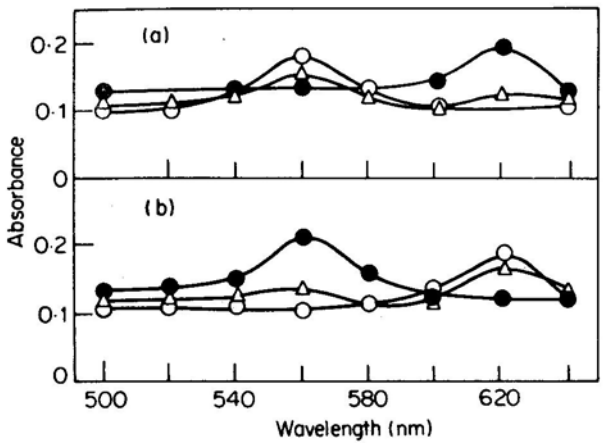


Figure 3. Formation of Phycocyanin and Phycocrythrin under different light quality conditions. Inoculum size same as in figure 1.10 ml of cell suspension extracted in 10 ml of distilled water.

(a) Water soluble pigments of fluorescent light grown culture transferred to incandescent light after 9days o-o, 12days Δ–Δ , 18days ●–●. (b) Water soluble pigments of incandescent light grown cultue transferred to fluorescent light after 9days o-o , l2days Δ–Δ, 18days ●–●.

light spectra and vice-versa. The ratio of phycoerythrin/phycocyanin, in fluorescent light grown culture is more than twice the value of the incandescent light green culture (table 1).

Table 1. Phycoerythrin and Phycocyanin ratios under different growth and light conditions.

Light treatment	PE/PC (560 nm/620 nm)			
	AA*	AA+N@	AA+G(L)†	AA+G(D)‡
Incandescent light (control)	0.68	0.66	0.72	0.90
Red light: 1 h	0.68	0.67	0.71	0.89
5 h	0.68	0.67	0.73	0.86
Green light: 1 h	0.78	0.72	0.76	0.92
5 h	0.81	0.78	0.80	0.98
Red:Green 2:2 h	0.80	0.70	0.82	0.90
Green:Red 2:2 h	0.70	0.66	0.74	0.88
Incandescent light (10 days)	0.67	—	—	—
Fluorescent light (10 days)	1.48	—	—	—

* AA: Allen and Arnon's medium.
@ AA+N: Supplemented with 20 mM potassium nitrate.
† AA+G(L): Supplemented with 1% glucose and grown in light.
‡ AA+G(D): Supplemented with 1% glucose and grown in dark.
PE: Phycoerythrin, PC: Phycocyanin.

With short light exposure (1-5 h), ending with green, phycoerythrin/phycocyanin increases and in red light exposures, this ratio decreases thereby showing that even short exposures to these light qualities affect the biliprotein synthesis (table 1). This ratio was nearly the same for glucose supplemented media and slightly less in nitrate supplemented media, although glucose given in the dark increases this ratio.

The main morphogenetic change is that in red light or incandescent light, the basal heterocysts tend to form pairs or occur as chains of three each although paired heterocysts rarely occur in intercalary positions. In fluorescent or green light, the basal heterocyst is single, just as in control culture, but intercalary paired heterocysts do occur in almost 10% of the filaments. The cell size gradient is almost the same in all treatments but in nitrate supplemented culture this gradient is absent as heterocysts are also absent and the formation of hormogonia is much more than the control. The sheath was nearly absent in such cultures (table 2). In glucose supplemented

Table 2. Morphogenetic changes under different conditions.

Treatment	Cell size gradient	Heterocyst position	Hormogonia	Sheath	Granulation	Polar plug	Branching	Colour of algal culture
Incandescent light	++	BPH+IH	50% (10-30C)	P	P	++	1%	Green-colour
Fluorescent light	++	BH+IPH(10%)	10% (8-20C)	P	–	+	–	Brown-colour
Incandescent +20mMKNO_3	–	–	80% (6-28C)	A	–	–	–	Green
Incandescent +1% glucose	+	BH+IPH(10%) BPH(2%F)	10% (6-20C)	P	–	–	1%	Green
Green light	++	BH+IPH(5%)	3% (6-20C)	P	–	+	–	Green brown
Red light	+++	BPH+IH(Rarely)	15% (8-26C)	P	+	+	0.5%	Green

– Absent; + Poorly present; ++ Present; + + + Distinct.
BH: Basal heterocyst; BPH: Basal Paired heterocyst; IPH: Intercalary paired heterocyst; IH: Intercalary heterocyst; F: Filaments C: Cells.
P: Present
A: Absent.

medium, in incandescent light, it formed basal heterocysts and paired intercalary heterocysts in about 10% of the filaments. Basal heterocysts in a chain of three have been observed in nearly 2% of the filaments. Next to heterocyst, 4-10 cells are quite bigger in size and appear bead shaped. Transfer of cultures to different light qualities often changed their mode of heterocyst formation and hormogonia production specifically.

Discussion

Various hypotheses have been proposed to explain the photocontrol of chromatic adaptation in blue-green algae (Fujita and Hattori, 1962; Diakoff and Scheibe, 1973; Bogorad, 1975; Björn and Björn, 1976; Tandeau de Marsac, 1977). The hypothesis proposed by Diakoff and Scheibe (1973) and Bogorad (1975) seems most attractive. It suggests that phycoerythrin/phycocyanin formation is controlled by photochemical

activity of a pigment independent of precursors of phycobiliprotein formation. Others suggest that there are precursors for both phycoerythrin and phycocyanin and the quality of light determines which type of biliprotein is to be formed (Fujita and Hattori, 1962).

The green light elicits production of phycoerythrin and red light produces phycocyanin. Similar is the case with fluorescent and incandescent light. In fluorescent light, the percentage of input lamp watts radiated in the green band is more than in the red band (Withrow and Withrow, 1956) whereas it is not the case in incandescent light spectrum, so there is induction in the synthesis phycoerythrin in fluorescent light.

According to Ohki and Fujita (1978), phycoerythrin formation had a 3-5 h lag period and then occurred almost linearly for 15-20 h until the formation slowed down and the pigment content reached a maximum level. We have found a maximum phycoerythrin/phycocyanin ratio of 1.5 and a minimum of 0.6, so that under the specific light conditions only one of these phycobilins predominates. With short exposures, values in between these two extremes were obtained thereby suggesting that there may not be any interconversion among the already formed phycocyanin and phycoerythrin, but only the precursors present at that time may have been induced by some independent pigment responding to the light quality (Diakoff and Scheibe, 1973; Ohki and Fujita, 1978). So there maybe some pigment functioning as phycochrome, similar to the phytochrome of higher plants, but with red:green reversal (see Shropshire, 1977). Besides the phycoerythrin induction, other photocontrolled phenomena are known to be involved in the growth of some blue-green algae (Bennett and Bogorad, 1973; Lazaroff, 1973; Diakoff and Scheibe, 1975; Tyagi and Ahluwalia, 1978). The observation of changes in heterocysts and hormogonia formation in the present study supports this hypothesis.

Acknowledgements

Thanks are due to Council of Scientific and Industrial Research, New Delhi, for the award of fellowships to ASA and RKR.

References

Allen, M. B. and Arnon, D. I. (1955) *Plant Physiol.,* **30,**366.
Benett, A. and Bogorad, L. (1973) *J. Cell Biol.* **58,** 419.
Björn, G. S. and Björn, L. O. (1976) *Physiol. Plant.,* **36,** 297.
Bogorad, L. (1975) *Annu Rev. Plant Physiol.,* **26,** 369.
Diakoff, S. and Scheibe, J. (1973) *Plant Physiol.,* **51,** 382.
Diakoff, S. and Scheibe, J. (1975) *Physiol. Plant,* **34,** 125.
Fujita, Y. and Hattori, A. (1960a) *Plant Cell Physiol.,* **1,** 281.
Fujita, Y. and Hattori, A. (1960b) *Plant Cell Physiol.,* **1,** 293.
Fujita, Y. and Hattori, A. (1962) *Plant Cell Physiol.,* **3,** 209.
Lazaroff, N. (1973) in *The biology of blue-green algae,* eds N. G. Carr and B. A. Whitton (Oxford: Blackwell Scientific Publications) p. 297.
Ohki, K. and Fujita, Y. (1978) *Plant Cell Physiol.,* **19,** 7.
Shropshire, W. Jr. (1977) in *The science of photobiology,* ed. K. C. Smith (London: Academic Press) p. 281.
Tandeau de Marsac, N. (1977) *J. Bacteriol.,* **130,** 82.
Tyagi, V. V. S. and Ahluwalia, A. S. (1978) *Ann. Bot.,* **42,** 1333.
Withrow, R. B. and Withrow A. P. (1956) *Radiai. Biol*., **3,** 125.