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The Effect of Light on the Content of Photosynthetically Active Pigments in Plants

IV. Chromatic Adaptation in Blue-green Algae *Anabaena cylindrica* und *A. variabilis*

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With 1 Figure

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Summary

CZECZUGA B. 1986. The effect of light on the content of photosynthetically active pigments in plants. IV. Chromatic adaptation in blue-green algae *Anabaena cylindrica* and *A. variabilis*. – Phyton (Austria) 26 (1): 1–9, 1 figure. – English with German summary.

The photosynthetic pigments (chlorophyll a, carotenoids and phycobiliprotein pigments) of two species of the genus *Anabaena* grown in white, red, yellow, green and blue light were examined.

The highest concentration of the cells was observed in the sample with red light in case of the both species, and the smallest with blue light.

The biggest amounts of chlorophyll a and carotenoids were included in the cells of samples with the yellow and smallest in case of the red light.

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The ratio of two phycobiliproteins is a follows:

– in *Anabaena cylindrica:* the highest amount of C-phycocyanin in the cells was observed in case of the red light, and C-phycoerythrin was found in the blue light.

– in *Anabaena variabilis:* the highest amount of C-phycocyanin in the cells was found in case of the yellow light, and allophycocyanin – was found in the blue light.

Zusammenfassung

CZECZUGA B. 1986. Einfluß des Lichtes auf den Gehalt photosynthetisch aktiver Farbstoffe in Pflanzen. IV. Chromatische Adaptation der Blaualgen *Anabaena cylindrica* und *A. variabilis*. – Phyton (Austria) 26 (1): 1–9, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Bei zwei Arten von Blaualgen der Gattung *Anabaena,* die bei weißem, rotem, gelbem, grünem und blauem Licht gezüchtet worden waren, wurde der Gehalt an photosynthetisch aktiven Farbstoffen (Chlorophyll a, Carotinoide und Phycobiliproteinfarbstoffe) untersucht.

Die größte Zellkonzentration wurde bei beiden Blaualgenarten bei rotem und die geringste bei blauem Licht beobachtet.

Der höchste Chlorophyll a- und Carotinoidengehalt wurde in den Zellen bei gelbem und der geringste bei rotem Licht festgestellt.

Das Verhältnis der Phycobiliproteinfarbstoffe gestaltete sich bei den erwähnten Blaualgen folgend:

– in den Zellen von *Anabaena cylindrica* wurde der höchste Gehalt an C-Phycocyanin bei rotem und der höchste Gehalt an C-Phycoerythringehalt bei blauem Licht gefunden,

– in den Zellen von *Anabaena variabilis* der höchste Gehalt an C-phycocyanin bei gelbem und der höchste Allophycocyaningehalt bei blauem Licht.

Introduction

As it known phycobiliproteins, chlorophyll a and carotenoids (O'CARRA & O'HEOCHA 1976) belong to the photosynthetically active pigments in the blue-green algae. The content of these pigment components of cell is changeable depending on the environmental conditions and first of all depending on the light conditions. Examinations on the contents of chlorophylls and carotenoids in green algae (CZECZUGA 1977, CZECZUGA & al. 1980) and in lichens (CZECZUGA 1981) cultivated at different wave length showed marked increase of chlorophylls and carotenoids in algae grown in green and blue light. This is one the forma of chromatic adaptation of these algae which allows them to develop in deeper layers of the water reservoirs where only shorter waves i. e. green and blue light penetrate.

Concerning observations on the influence of green and blue light on the amount of chlorophyll and carotenoids in green algae it is interesting to notice the influence of different wave length on the content of photosynthetically active pigments in blue-green algae which as we know have different pigment components in comparison to algae. Besides measurements of the examined cells of blue-green algae cultured at different wave length and production of heterocysts were carried out.

Material and methods

Examinations were carried out on two species i. e. *Anabaena cylindrica* LEMMERMANN and *Anabaena variabilis* KÜTZING ex BORNET et FLABARIET cultivated in the Department for a long time. The culture of *Anabaena cylindrica* comes from the Laboratory of Algal Physiology, University of Texas (USA) and the culture of *A. variabilis* comes from Kyoto College of Pharmacy (Japan). Cultures of the examined species were carried out in 250 ml bottles with tap water. The bottles were put into special boxes with the appropriate glass filtres (CZECZUGA 1977, 1981). The glass was manufactured by the FPN- Bytom Works, wavelengths being indicated by the producers. Four basis colours were put to use: red (λ = 700 nm), yellow (λ = 590 nm), green (λ = 500 nm) and blue (λ = 450 nm). A culture of each species investigated grown in a box provided with usual, "colourless" (white), glass served for control. The cultures were shaken twice a day. For illumination served glow tube lamps, the intensity of incident light war 1650 lux. according with 0.004 W · m^{-2}. The dayly light – dark – period amounted to 10 : 14. Samples were taken from each bottle to evaluate the cell concentration after 21 days of the experiment. The evaluation was carried out by means of Thoma camera with simultaneous counting of the number of heterocysts (trichomes) and measuring the cell length and width.

The remaining culture from each bottle was divided into two parts which were filtered through the membrane filters to determine phycobiliprotein pigments, chlorophyll a and carotenoids. The presence of particular phycobiliproteins was determined using the following method.

The phycobiliprotein was extracted from the homogenized material at a low temperature for 1 hour with 0.01 M phosphate buffer with 0.15 M NaCl at pH 7.0. It was then centrifuged at 4° C in a K-24 Janetzki centrifuge at 20,000 × g for 10 min. The precipitate obtained was saturated to 35% (fraction I) with ammonium sulphate and left for 2 hr after which it was centrifuged again at 22,300 × g for 15 min. The supernatant was saturated to 70% (fraction II) with ammonium sulphate, left for 1 hr, and the precipitate thus formed was centrifuged at 22,300 × g for 15 min. Both fractions were then suspended in 6 ml of 0.1 M phosphate buffer pH 7.0 and dialyzed overnight in a Dialysia Membrane (made by the Union Carbide Corporation, Chicago USA) at 4° C in the presence of the same phosphate buffer at pH 7.0.

Both fractions were then applied to a Sephadex G-100 column (made by Pharmacia Fine Chemicals AB, Uppsala, Sweden) previously washed in 0.1 M phosphate buffer pH 7.0.

Fraction I, containing phycoerythrine.

The phycoerythrine was washed of the column a 0.1 M phosphate buffer, then saturated to 30% with ammonium sulphate, left for 1 hr, and centrifuged at 4° C and 22,300 × g for 30 min. The precipitate was dissol-

ved in 0.1 M phosphate buffer pH 7.0 to which a few drops of 0.01% sodium azide was added to prevent bacterial growth. After overnight dialysis at 4° C in the presence of the same buffer, measurements of extinction were made.

Fraction II, containing phycocyanine and allophycocyanine.

After passing throught the column, phycocyanine was washed of with 400 ml of phosphate buffer in a linear gradient of concentration of 0.005–0.1 M (allophycocyanine remains at the highest point). The phycocyanine was saturated with ammonium sulphate to 65%, left for 1 hr and then centrifuged at 14,000 × g for 15 min. The precipitate was suspended in a small volume of 0.1 M phosphate buffer pH 7.0 containing 0.01% of sodium azide and dialyzed overnight at a low temperature in the presence of the same buffer. After dialysis the extinction was measured.

For elution allophycocyanine from the column, a 0.5 M phosphate buffer pH 7.0 was used. The eluent was then saturated to 75% with ammonium sulphate, left overnight, and then centrifuged at a low temperature and 22,300 × g for 30 min. The precipitate obtained was suspended in a small volume of 0.1 M phosphate buffer pH 7.0 containing 0.01% sodium azide and dialyzed overnight in the presence of the same buffer after which the extincton measurements were made.

The absorption maxima of the isolated fractions were determined on a Spektromom – 203 spectrophotometer.

The total phycobiliprotein pigment content of the blue-green algae and the percentages of content of the various pigment were determined by the method described in the paper of BENNETT & BOGORAD (1973).

The total contents of chlorophyll and carotenoids were determined following JEFFREY & HUMPHREY (1975).

Results

Fig. 1. presents concentration of cells in one milliliter of the culture of *Anabaena cylindrica* and *A. variabilis*. The highest concentration of the cells was observed in the sample with white filter in case of the both species. Concerning the coloured filters the highest concentration was again observed in case of the both species in the cultures with the red filter, and the smalles concentration in the samples with the blue filter.

Table 1 presents results of the cell measurements and the mean content of heterocysts in the thread of each of the examined species of *Cyanophyceae*. The longest cells were observed in the cultures with the green filter and the shortest in the cultures with the blue filter both in case of *Anabaena cylindrica* and *A. variabilis*. And the widest cells in the both species were found in the samples with the red filter and it was bigger then in the samples

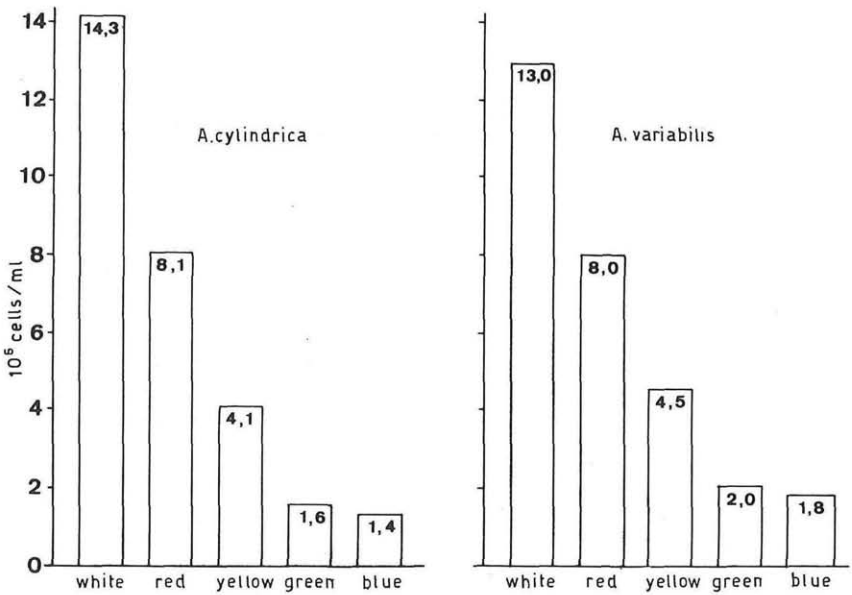

Fig. 1. Number of cells of the both investigated algae species cultivated in white, red, yellow, green and blue light.

with the white filter. Besides the mean number of heterocysts in one thread was also the biggest in the samples with the red filter, it was slightly smaller in the samples with the white filter and in the other samples it was remarkably different in comparison to the samples with the red filter (Table 1).

Table 1

Mean of size of cells (μm) and mean number of heterocysts in *Anabaena cylindrica* and *Anabaena variabilis* grown in white, red, yellow, green and blue light (n = 100)

	white	red	yellow	green	blue
A. cylindrica					
length of cell	3.6	4.1	4.1	4.3	3.4
width of cell	2.4	2.9	2.4	2.4	2.5
number of heterocysts	1.8	1.95	0.80	0.50	0.65
A. variabilis					
length of cell	3.8	4.3	4.3	4.5	2.8
width of cell	2.7	3.2	2.9	2.9	3.0
number of heterocysts	2.20	2.25	1.30	1.45	1.70

Results of the analysis of chlorophyll a and of carotenoids content are presented in Table 2. The results show that the biggest amounts of chlorophyll a and carotenoids were included in the cells of samples with the yellow filter and slightly less in case of the green filter. The smalles amount of the pigment was included in the cells of samples with the red filter, the amount was even smaller then in case of the white filter. It is especially clear

Table 2

Pigment content of *Anabaena cylindrica* and *Anabaena variabilis* grown in white, red, yellow and blue light. Absolute pigment content in µg per 10^6 cells, relative values (in parentheses) in %.

	white		red		yellow		green		blue	
	µg	%	µg	%	µg	%	µg	%	µg	%
Anabaena cylindrica										
chlorophyll a	0.200	(100.0)	0.189	(94.5)	0.500	(250.0)	0.487	(243.5)	0.426	(213.0)
carotenoids	0.125	(100.0)	0.103	(82.4)	0.289	(231.2)	0.288	(230.4)	0.191	(152.8)
ratio $\frac{\text{chlorophyll}}{\text{carotenoids}}$	1.6		1.8		1.7		1.7		2.2	
Anabaena variabilis										
chlorophyll a	0.254	(100.0)	0.173	(68.1)	0.418	(164.6)	0.415	(163.4)	0.308	(121.3)
carotenoids	0.158	(100.0)	0.101	(63.9)	0.260	(164.6)	0.234	(148.1)	0.158	(100.0)
ratio $\frac{\text{chlorophyll}}{\text{carotenoids}}$	1.6		1.7		1.6		1.8		1.9	

Table 3

Phycobiliprotein pigments content of *Anabaena cylindrica* and *Anabaena variabilis* grown in white, red, yellow, green and blue light. Absolute pigment content in µg per 10^6 cells, relative values (in parentheses) in %.

	white		red		yellow		green		blue	
	µg	%	µg	%	µg	%	µg	%	µg	%
A. cylindrica										
C-phycocyanin	0.321	(100.0)	0.996	(300.9)	0.365	(113.7)	0.773	(240.8)	0.896	(279.1)
C-phycoerythrin	0.107	(100.0)	0.142	(132.7)	0.063	(58.8)	0.129	(120.5)	0.430	(401.8)
A. variablilis										
C-phycocyanin	0.958	(100.0)	0.825	(86.1)	1.048	(109.3)	1.027	(107.2)	0.868	(90.6)
Allphycocyanin -B	0.176	(100.0)	0.586	(332.9)	0.094	(53.4)	0.159	(90.3)	1.162	(660.2)
C-phycoerythrin									0.578	(578.0)

when presented in percentage (Table 2). Results concerning phycobiliprotein pigments presented in Table 3 show incidence of C-phycocyanin and C-phycoerythrin in the cells of *Anabaena cylindrica*. The percentage ratio of these two phycobiliproteins is as follows: the highest percenta- of C-phycocyanin in the cells was observed in case of the red filter (87.2%) and the highest percentage of C-phycoerythrin was found in the cells cultivated with blue filter (32.4%).

C-phycocyanin and allophycocyanin -B were observed in the cells of *A. variabilis* cultivated with the white red, yellow and green filters. And in the case of the blue filter C-phycoerithrin was also observed besides C-phycocyanin and allophycocyanin-B. The highest percentage of C-phycocyanin was found in the cells of *A. variabilis* cultivated with the yellow filter (91.8%) and allophycocyanin-B with the blue filter (44.5%). The content of C-phycoerythrin in the cells when using the filter amounted to 22.2% of the total content of phycobiliproteins.

Discussion

The highest concentration of cells of *Cyanophyceae* observed in cultures with the filter was also demonstrated for other species of algae. It mainly concerns such unicellular green algae as species of the genera *Chlorella* and *Scenedesmus* (CZECZUGA 1977, CZECZUGA & al. 1980). Yet in case of the above mentioned green algae the lowest concentration of cells in one milliliter of the culture was observed when using the green filter but in case of the examined *Anabaena* species the lowest concentration of cells was observed when using the blue filter. Using the red filter resulted in the widest cell measurements of the examined blue-green algae and the cells included the biggest amounts of heterocysts. It is worth mentioning that AHLUWALIA & KUHMAR (1980) showed the red and orange light to induce the spore germination in *Nostoc ellipsosporum*.

The phycobiliprotein pigments C-phycocyanin and C-phycoerythrin found in the cells of *Anabaena cylindrica* and C-phycocyanin and allophycocyanin-B found in the cells of *Anabaena variabilis* were also demonstrated by other authors (HATTORI & FUJITA 1959, FUJITA & TSUJI 1968, CHAPMAN & al. 1967, ŁOŚ 1980, CZECZUGA 1982). It should be noted C-phycoerythrin was also observed besides C-phycocyanin and allophycocyanin-B in the cells of *Anabaena variabilis* cultivated with the blue light. It should be added that RÜDIGER & al. (1980) cultivating *Pseudanabaena catenata* with the red and green light showed the presence of C-phycoerythrin if the cells of the examined blue-green algae were growing in the green light but there was no C-phycoerythrin in the cells growing in the red light. It is known (HATTORI & FUJITA 1959, FUJITA & HATTORI 1960, ZEWNIER & al. 1965) that the red-orange light stimulates phycocyanin biosyntheis but the green-blue light and first of all green light stimulate the growth of phycoerythrin. This is the phenomenon of so called chromatic adaptation of blue-green algae (BOGORAD 1975). In our examinations the highest concentration of phycoerythrin was found in the cells of *A. cylindrica* and *A. variabilis* cultivated only with the blue filter. And the highest concentration of phycocyanin was observed in *A. cylindrica* cultivated with the red filter but in case of *A. variabilis* – with the yellow filter. BENNETT & BOGORAD (1973) showed decrease of phycoerythrin in the cells of *Fremyella diplosiphon* growing in

the red light. Is should be noted that according to other authors two of the three spectra which stimulate the phycocyanin biosynthesis in *Cyanidium caldarium* are found to be in the range of 375 and 645 nm (SCHNEIDER & BOGORAD 1979) and in case of *Fremyella diplosiphon* – in the range 575 and 645 nm (HAURY & BOGORAD 1977, VOGELMANN & SCHEIBE 1978).

The light intensity also effects the contents of particular phycobiliprotein pigments. When the light is weak the cell concentration of pigments of the phycoerythrin group increases (JUPIN & al. 1980, FUGLISTALLER & al. 1981) or the number of phycobiliprotein structures in cells increases (VIERLING & RANDALL 1980).

Chlorophyll a and carotenoids were found in the biggest amounts in the cells of the examined blue-green algae when cultivated with the yellow and green filter. It should be added that the highest concentration of these pigments was observed in green algae of the genera *Chlorella* and *Scenedesmus* when cultivated with the green filter (CZECZUGA 1977, CZECZUGA & al. 1980).

The examination shows that blue-green algae have the ability of chromatic adaptation to the wide range of the light rays. The red light stimulated the phycocyanin growth, the yellow and green light stimulated the growth of chlorophyll a and carotenoids and the blue light stimulated the growth of phycoerythrin. It should be supposed that besides the adaptation mechanisms to the light conditions described in this paper there are also other adaptation mechanisms.

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