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Effect of carbon dioxide concentration on pigmentation in the blue-green alga *Anacystis nidulans*

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The pigment content in the blue-green alga *Anacystis nidulans* was found to be dependent upon CO_2 concentration during growth. In cells grown with 1% CO_2 in air the total pigment constituted 20.5% of the dry weight while it was only 11.1% of dry weight of cells grown in air (0.03% CO_2). This decrease in total pigment was found to be almost entirely ascribable to decrease in phycocyanin. Since light absorbed by phycocyanin has been shown to provide nearly equal rates of photoreactions I and II, the "CO_2 control" of phycocyanin is viewed as an effective means of regulation of the photoreactions without upsetting the balance of operation of the two photoreactions.

Pigmentation studies on the blue-green alga *Anacystis nidulans* grown under various light intensities of white light have revealed the operation of the so-called "sun-and-shade phenomenon" in which pigment content varies inversely with light intensity (*1*). Such changes in pigment density allow cells to increase their pigment content under low light intensity in order to capture more of the available light. Myers and Kratz (*1*) demonstrated this increase in pigmentation for both chlorophyll *a* and for the primary accessory pigment, phycocyanin, with no significant change in the chlorophyll/phycocyanin ratio.

Jones and Myers (*2*) observed that *Anacystis* cells grown in red light, which provided excess quanta to chlorophyll, had a decrease in chlorophyll content to about one-fourth with no significant change in the content of the phycocyanin or the total carotenoid. These investigators interpreted this as a mechanism to prevent pigment system 1 from receiving quanta in excess, and thus maintaining the balance of energy input to both of the pigment systems.

It has thus been shown that *Anacystis nidulans* can change its pigmentation in response to changes in light intensity and wavelength in order to allow the maximum light capture and to maintain a balance of quanta input to the two photoreactions. The present report describes changes in the pigment composition of *Anacystis nidulans* caused, not by the wavelength of light or its intensity, but by CO_2 concentration during growth.

Materials and methods

Anacystis nidulans Drouet (Kratz and Allen strain) was cultured in medium C of Kratz and Myers (*3*) in a continuous culture apparatus (*4*) at 37°C. Illumination

was provided by six 20-w cool white fluorescent lamps surrounding the chambers and providing approximately 400 ft-candles (approximately 13×10^3 ergs/cm^2/sec from 300 to 800 nm) at the culture surface. Aeration was with air or with 1% CO_2 in air obtained from a gas-mixer. The CO_2-chamber was harvested at 12 or 24-hr intervals by draining two-thirds of the suspension and adding fresh sterile medium to the remaining cells. The air-chamber was treated in a similar manner except the culture was harvested at 2 to 4 day intervals. The specific growth rate (*1*) in air (0.15) was approximately one-tenth of that obtained in the presence of 1% CO_2 (1.44).

Pigment analyses were based on OD measurements, obtained in a Beckman DB-G spectrophotometer with ten-inch recorder, using whole cell suspensions, aqueous extracts after sonication, and the 80% acetone extracts as described previously by Myers and Kratz (*1*). Four 25 ml aliquots of freshly harvested suspension were centrifuged and washed once with distilled water. After a second centrifugation of the washed cell suspension, one aliquot was quantitatively transferred to a preweighed tare, dried to constant weight and reweighed for dry weight determination. A second aliquot was resuspended in a known volume of water and used for determination of the whole cell absorption spectra from 400 to 730 nm. White translucent lucite (Cadillac Plastics No. W-7328) immersed in the cuvettes served to minimize scattering by the Shibata technique (*5*). The OD reading at 730 nm was substracted from all wavelengths as a uniform scattering correction. A third aliquot was resuspended in 80% acetone for 5 min and centrifuged. After a second and third extraction the supernatants containing the chlorophyll and carotenoid were combined and made up to known volume to obtain the absorption spectra. The blue cells remaining after extraction with 80% acetone showed a characteristic phycocyanin absorption with no trace of chlorophyll or carotenoid absorption. A fourth aliquot was resuspended in water and subjected to sonication for 10 min in a Biosonic III cell disintegrator, followed by centrifugation at $10{,}000 \times g$ for 10 min. The clear blue-green supernatant was made up to known volume and its absorption spectrum was determined.

Results

Fig. 1 shows a typical absorption spectrum of intact cells from *Anacystis nidulans* grown under air or 1% CO_2 in air. The traces represent the absorbance of a 1.0 cm layer containing 1.0 mg dry weight of cells per ml. Cell density at harvest was 0.32 mg dry weight per ml for the CO_2-grown cells and 0.26 mg dry weight per ml for the air-grown cells. At these nearly equal cell densities, the effective light intensities are approximately equal and therefore the pigmentation differences are not due to differential absorption of light quanta.

The pigmentation differences for the two cell types shown in Fig. 1 can be clearly seen with the unaided eye: CO_2-grown cells appear dark blue-green while air-grown cells are yellow-green and might easily be mistaken for a culture of green algae. As a first approximation for pigment variation, the ratio of whole cell absorption peaks at 678 nm (chlorophyll), 625 nm (phycocyanin) and 490 nm (carotenoids) may be compared for air- and CO_2-grown cells. As shown in Table 1, CO_2-grown cells show a chlorophyll/phycocyanin absorption ratio of 0.80 and a

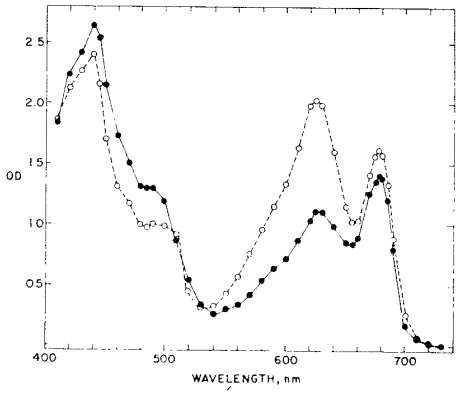

Fig. 1. *Absorption spectra for cells grown with air (solid line) and cells grown with 1% CO_2 in air (broken line).* Growth under 400 ft-candles of white light at 37°C. Curves are adjusted to give OD of a 1.0 cm layer containing 1.0 mg dry weight of cells per ml. Scattering was minimized by use of 3 mm lucite diffusion plates.

carotenoid/phycocyanin absorption ratio of 0.50 which agree closely with values reported by Jones and Myers (*2*) for CO_2-grown cells. Air-grown cells, however, show a marked decrease in the phycocyanin peak at 625 nm and exhibit an increase in the carotenoid peak at 490 nm. Thus, cells grown under low CO_2 have chlorophyll/phycocyanin and carotenoid/phycocyanin absorption ratios of 1.27 and 1.17 respectively.

More detailed analyses of the pigmentation differences were made on data obtained on the extracted pigments. Fig. 2A shows the absorption spectra of 80% acetone extracts from the two cell types. The spectra differ primarily in increased absorption by carotenoids (480 nm) in the case of air-grown cells. Fig. 2B shows the absorption spectra of the total aqueous extracts after sonication. Here the extracted pigments show a major decrease in phycocyanin absorption (618 nm) for air-grown cells.

Concentrations of chlorophyll *a* and total carotenoid were estimated in the 80% acetone extracts (*1*), using extinction coefficients of 82.04 at 663 nm and 200 at 460 nm for chlorophyll and total carotenoid respectively (*6*). Estimation of phycocyanin concentration was made on the aqueous extracts by use of the following equation in which correction is made for chlorophyll absorption (*1*):

$$\text{OD phycocyanin} = 1.016\ OD_{618} - 0.203\ OD_{677}$$

Table 1
Effects of CO_2 on pigment composition

Growth conditions	Relative peak heights[a]		Pigment concentrations in % dry weight				Pigment ratios
			Chl[b]	Car[c]	Phy[d]	Total	Chl : Phy : Car
	678/625	490/625					
1% CO_2	0.80	0.50	1.97	0.75	17.7	20.5	1 : 9.0 : 0.4
Air	1.27	1.17	1.79	1.05	8.2	11.1	1 : 4.6 : 0.6

[a] OD ratios at the wavelengths shown in nm as obtained on whole cell suspensions.
[b] From OD measurements at 663 nm on 80% acetone extracts; % chlorophyll = $OD_{663}/0.8024$.
[c] From OD measurements at 460 nm on 80% acetone extracts; % carotenoid = $OD_{460}/2.0$.
[d] From OD measurements at 618 nm and 677 nm on aqueous extracts; % phycocyanin = $1.016\ OD_{618} - 0.203\ OD_{677}/0.079$.

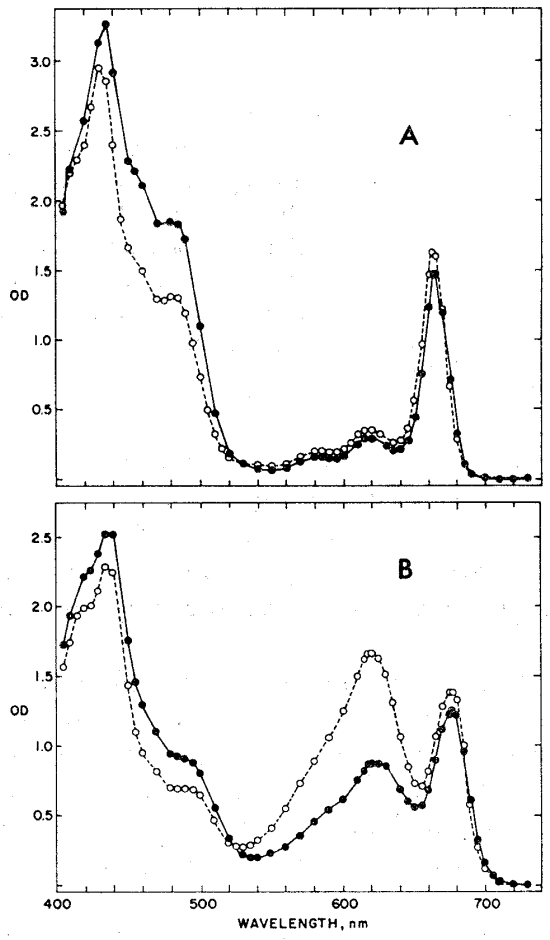

Fig. 2. *Absorption spectra of extracts from cells grown with air (solid line) and cells grown with 1% CO_2 in air (broken line).* A: 80% acetone extracts. B: aqueous extracts after sonication. Curves are adjusted to give OD of a 1.0 cm layer containing 1.0 mg dry weight of cells per ml.

Phycocyanin content is then determined from the specific absorption coefficient of 7.9 at 618 nm (*7*).

Results, given in Table 1, show total pigment content of 20.5% of the dry weight for CO_2-grown cells *versus* 11.1% for air-grown cells. With CO_2-grown cells of *Anacystis*, Myers and Kratz (*1*) reported 22.0% of the dry weight as pigment. From their raw data one may calculate a ratio of chlorophyll *a* to phycocyanin as 1 : 9.3 and chlorophyll *a* to carotenoid as 1 : 0.4. These values are very close to our own observations in which we find a ratio of chlorophyll *a* to phycocyanin as 1 : 9.0 and that of chlorophyll *a* to carotenoid as 1 : 0.4. In contrast, when grown in air, *Anacystis* shows a reduction in phycocyanin content so that the ratio of chlorophyll *a* to phycocyanin is reduced to 1 : 4.6; in six separate experiments the ratio was always within 1 : 4.5 to 1 : 4.7. Moreover, the reduction in phycocyanin content is primarily responsible for the decrease in total pigment as observed in air-grown cells.

As medium C is poorly buffered, it seemed possible that the pigment differences between the air and CO_2-grown cells might in reality be a pH effect. In medium C,

the air-grown cultures had, at harvest, a pH between 8.9 and 9.3 while the pH of CO_2-grown cells was maintained near 8.0. To increase the buffering capacity, 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) was added to the medium. The addition of HEPES maintained the pH of air-grown cells at 8.0–8.2 but the pigmentation remained unaltered. We therefore suggest that the pigment differences between the air and the CO_2-grown cells is a CO_2 effect and not a pH effect.

Discussion

The complex control of pigmentation in algae in response to illumination intensity and wavelength has received recent attention by Jones and Myers (*2*) who reported only small variations in the ratio of absorptions for chlorophyll/phycocyanin under white light illumination. Their data suggest that *Anacystis nidulans* has a sufficient balance of chlorophyll and phycocyanin absorptions and the authors suggest that no serious change in this absorption ratio should occur by any illumination likely to be experienced in nature or by any change in cell concentration. Their findings are also in agreement with the earlier findings of Myers and Kratz (*1*) who observed a decrease in the total pigment caused by a ten-fold increase in light intensity but found only minor changes in the pigment ratios. Jones and Myers (*2*) found a change in the ratio only in the special case of illumination by red light, and the change was in the direction of restoring the chlorophyll/phycocyanin absorption ratio. Ghosh and Govindjee (*8*) have also reported similar changes in pigment ratios in *Anacystis* caused by illumination with light of different colors.

The presently described "CO_2 control" is viewed as a means of regulation of quanta input to maintain the photoreactions at a rate comparable with the dark reactions. The fact that the air-grown cells were limited in growth by the availability of CO_2 is revealed by a specific growth rate approximately ten-fold lower than that of the CO_2-grown cells. Air-grown cells were thus limited by the dark reactions of photosynthesis and not by the light reactions which received quanta in excess of that required for the maintenance of the dark reactions. Air-grown cells might therefore reduce their pigment content in order to effect the reduced quanta input comparable to the limiting dark reactions. Indeed, our results indicating a total pigment content of 20.5% of the dry weight for CO_2-grown cells *versus* 11.1% for air-grown cells support this hypothesis. Such a control is similar to the "sun-and-shade control". There is, however, one major difference between the "sun-and-shade control" of pigmentation and the presently described "CO_2 control". In the former, the pigmentation changes occur without affecting the ratio of chlorophyll to phycocyanin, presumably maintaining a balance of quanta input to drive the two photoreactions. The "CO_2 control", in contrast, involves pigmentation changes which result in alteration of the ratio of chlorophyll to phycocyanin. As shown in Table 1, the low pigmentation in air-grown cells is almost entirely ascribable to a reduction in the phycocyanin content and not to a uniform decrease in all of the pigments.

We must turn therefore to the question of quanta distribution in *Anacystis* as investigated from Emerson enhancement studies. Enhancement data obtained on CO_2-grown cells have shown large enhancement values in the regions of maximum

chlorophyll absorption indicating that absorption energy is supplied in excess to photoreaction I (*9*).

In the region of maximum phycocyanin absorption, however, enhancement values are very low indicating that the two light reactions are closely balanced. Such low enhancement values lead to the suggestion that phycocyanin may absorb quanta for each of the photoreactions and thus may provide a balance of operation between them.

The present work appears to lead toward a conclusion that the "CO_2 control" of pigmentation acting through phycocyanin might allow adjustment of the light reactions to match the dark reactions by which the CO_2 is fixed and reduced, and might do so without upsetting the balance between the two photoreactions.

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