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# Relationship Between Age of Culture and Occurrence of the Pigments of Photosystem II of Photosynthesis in Heterocysts of a Blue-Green Alga

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Microspectrophotometric examination of the pigments in vivo of heterocysts of *Anabaena* sp. L-31 has shown that most heterocysts of 2-day-old cultures possess only very small amounts, if any, of c-phycocyanin, allo-phycocyanin, and c-phycoerythrin, the main pigments comprising photosystem II of photosynthesis. The quantities of these pigments, however, increase with age of cultures, and by the end of 5 days the majority of heterocysts contain comparatively large amounts. The culmination of this sequential development is observed in most heterocysts of 7- to 15-day-old cultures when the full complement of photosystem II pigments is present. The spectral characteristics at this stage are similar to those of vegetative cells and suggest a dedifferentiation of heterocysts.

Heterocysts, the differentiated cells of many filamentous blue-green algae, have been implicated as the sites of nitrogen fixation in these organisms (3). Stewart et al. (8) and Wolk and Wojciuch (14) obtained strong biochemical evidence in support of this contention. Thomas and David (11) found that depletion of cellular ammonia induces heterocyst production possibly to promote fixation of molecular nitrogen in order to restore the ammonia level. Others (4, 6, 7, 9), however, have been able to induce high nitrogenase activity in vegetative cells under anaerobic conditions.

Nitrogenase activity is known to be inhibited by oxygen, and it was suggested (3) that heterocysts lack a functional photosystem II of photosynthesis responsible for the evolution of molecular oxygen. Subsequently, it was shown (2, 13) that, compared to normal cells, heterocysts have very little c-phycocyanin, the principal pigment of photosystem II. Thomas (10) demonstrated the absence in vivo of all the major pigments of photosystem II (chlorophyll *a* 670, c-phycocyanin, c-phycoerythrin, and allophycocyanin) in young heterocysts of *Anabaena* sp. L-31. Here, we report results of a detailed in vivo microspectrophotometric examination of heterocysts of *Anabaena* sp. L-31 which show that the pigment composition of heterocysts is dependent on the age of culture and that an increasing proportion of older heterocysts contains pigments comprising photosystem II.

## MATERIALS AND METHODS

*Anabaena* sp. L-31 was grown in 500-ml conical flasks in Myer's medium (5) modified to promote the growth of blue-green algae (1). Temperature was maintained at 28 ± 1 C. Light intensity was 5,000 lux, and the cultures were 5 cm deep. The cultures were aerated at a rate of 5 liters/min. Initial optical density of the cultures was adjusted to 0.05 at 450 nm, with inoculum from 15-day-old cultures.

Samples were withdrawn at 24-hr intervals until day 5, and then on days 7, 10, and 15. The pigment composition was examined, as reported earlier (10, 12) by using a Universal microspectrophotometer (UMSP I, Zeiss). Well differentiated heterocysts were observed in large numbers only after 48 hr. More than 100 such heterocysts, characterized by the presence of a thick wall and distinct polar bodies, were selected at random from each sample for study of their spectral properties. Measurements were made on an area having 6-$\mu$m diameter which would encompass whole heterocysts.

The absorption maxima in vivo of the major pigments of *Anabaena* sp. L-31 are 682, 666, 650, 610 to 620, and 580 nm, for the long-wavelength form of chlorophyll *a* (C *a* 680), the short-wavelength form of chlorophyll *a* (C *a* 670), allophycocyanin (allo-PC), c-phycocyanin (c-PC), and c-phycoerythrin (c-PE), respectively (10). The broad peak near 490 nm shows

carotenoid absorption and the maxima near 440 and 420 nm are the Soret bands of chlorophyll *a*. The changes in peak heights at these wavelengths indicate quantitative variations of these pigments. The spectra obtained each day from measurements on heterocysts were examined for such changes and were found to fall into eight distinct categories mainly according to the quantitative changes in the absorption due to C *a* 670, allo-PC, c-PC, and c-PE. The per cent distribution of these spectral types were then tabulated for algal samples of different ages.

## RESULTS

The different types of absorption spectra obtained from heterocysts of 2-day-old cultures are presented in Fig. 1, along with the typical spectrum of a vegetative cell. Spectrum I shows the absence of peaks attributable to C *a* 670, c-PC, allo-PC, and c-PE. This spectrum is typical of very young heterocysts which lack the entire complement of pigments comprising photosystem II. Moreover, the amount of the long-wave form of chlorophyll *a* (C *a* 680) present is less than that found in other spectral types. In type II spectra the peak height of chlorophyll *a* is enhanced. c-PC appears as a hump, though the peak height is always much less than that of chlorophyll *a*. Type III spectra show greater amounts of c-PC; the peak of c-PC, however, still subtends that of chlorophyll *a*. In type IV spectra, chlorophyll and c-PC peak heights are equal. Meanwhile, the trough between chlorophyll and c-PC peaks becomes less deep, indicating the appearance of allo-PC (10). c-PE can also be detected in these spectra by the change of slope of absorption near 580 nm.

A different set of spectral properties, in addition to that observed in 2-day-old cultures, are found in heterocysts of older (3–15 days) cultures (Fig. 2). These spectra are characterized by the presence of increasing amounts of c-PC, c-PE, and allo-PC. Meanwhile the wavelength of the absorption peak of chlorophyll *a* shifts from 680 to 670 nm, indicating the appearance of the short-wave form of chlorophyll *a* (10). These changes depict the emergence of the full complement of the pigments of photosystem II. In types VII and VIII, the spectra are indistinguishable from that of young vegetative cells.

The per cent distribution of the different spectral types in heterocysts of cultures of different ages is presented in Table 1. In 2-day-old cultures, heterocysts with spectral types I, II and III predominate, while types V to VIII are absent. In 3-day-old cultures, type IV spectra appear conspicuously. By day 5 (4-day-old cultures) heterocysts with spectral types I and II decrease rapidly and are replaced by types IV and V. In cultures which are 7 days old, type V becomes the predominant spectral feature. There is also a significant percentage of types VI and VII heterocysts. The culmination of this age-dependent development of the pigments of photosystem II is observed in 15-day-old cultures where the majority of heterocysts belong to spectral types V to VII.

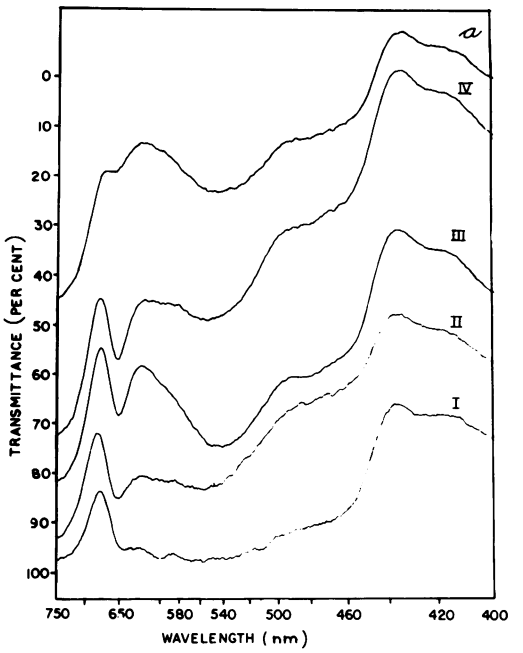


FIG. 1. *In vivo absorption spectra (I to IV) of heterocysts of 2-day-old cultures of Anabaena sp. L-31. Spectrum a is typical of vegetative cells of the alga. Spectra II, III, IV, and a are superposed by 5, 17, 27 and 55 divisions, respectively, in percentage transmittance scale to facilitate comparison. See text for details.*

The results also show that the long-wave form of chlorophyll *a* (C *a* 680) and carotenoids which mainly constitute photosystem I are invariably present in heterocysts at all stages of development.

## DISCUSSION

Thomas et al. (10; and J. Thomas, K. A. V. David, and A. R. Gopal-Ayengar, First International Symposium on Taxonomy and Biology of Blue-Green Algae, Centre for Advanced Study in Botany, University of Madras, Madras, India, January, 1970, Proceedings, *in press*) analyzed the spectral characteristics of vegetative cells of *Anabaena* sp. L-31 and showed a sequential reduction and disappear-

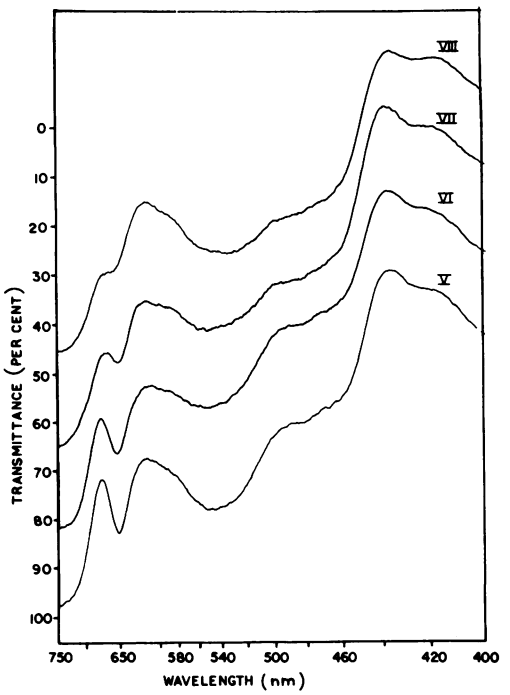


FIG. 2. *In vivo absorption spectra (V to VIII) of heterocysts of 3- to 15-day-old cultures of Anabaena sp. L-31. Spectra VI, VII, and VIII are superposed by 14, 28, and 45 divisions, respectively, in percentage transmittance scale to facilitate comparison. See text for details.*

TABLE 1. *Per cent distribution of specific spectral types[a] in heterocysts of Anabaena sp. L-31*

| Age of culture (days) | Spectral types (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII |
| 2 | 28 | 29 | 41 | 2 | 0 | 0 | 0 | 0 |
| 3 | 20 | 7 | 44 | 20 | 7 | 2 | 0 | 0 |
| 4 | 8 | 10 | 47 | 22 | 12 | 1 | 0 | 0 |
| 5 | 4 | 14 | 34 | 34 | 12 | 2 | 0 | 0 |
| 7 | 9 | 17 | 20 | 18 | 24 | 8 | 3 | 1 |
| 10 | 9 | 15 | 34 | 12 | 18 | 10 | 2 | 0 |
| 15 | 5 | 1 | 12 | 16 | 38 | 18 | 10 | 0 |

[a] Spectral types were determined mainly according to the changes in absorption (increasing order of absorption from type I to VIII) due to chlorophyll *a* 670, allo-phycocyanin, c-phycocyanin, and c-phycoerythrin. See Fig. 1 and 2, and text for details.

ance of the pigments comprising photosystem II eventually giving rise to the spectral properties of young heterocysts. The present results indicate that with aging there is a reversal of this process in heterocysts resulting in the restoration of the full complement of the pigments of photosystem II. This is suggestive of dedifferentiation of heterocysts.

If, as expected, molecular oxygen produced by photosystem II inhibits nitrogenase activity, there should be maximum activity in 2-day-old cultures and decreasing activity thereafter. We have results to suggest that this in fact is the case. Quantitative cytochemical examination of heterocysts from cultures of different ages with a microspectrophotometric technique (1) and parallel studies on the effect of age of culture on acetylene reduction (Thomas and David, *unpublished data*) indicate a definite relation with the appearance of photosystem II pigments and decrease in nitrogenase activity in heterocysts of *Anabaena* sp. L-31. However, recent reports of nitrogenase activity in the nonheterocystous alga *Plectonema boryanum* (4, 9) and vegetative cells of *Anabaena cylindrica* (4, 6, 7) suggest that the enzyme activity is induced in vegetative cells in the absence of or under low tension of oxygen. Nevertheless, our results (1) as well as those of Wolk and Wojciuch (14) suggest that heterocysts are the preferred sites of nitrogenase activity during active aerobic photosynthesis.

## ACKNOWLEDGMENT

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