Pigment distribution in cyanobacteria: An in vivo microspectroscopic investigation

L. Barsanti, V. Passarelli and P. Gualtieri

C.N.R., Istituto di Biofisica, Via S. Lorenzo 26, I-56 100 Pisa (Italy)
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Summary. In vivo microspectroscopy represents an effective and reliable technique to study pigment composition and distribution. In contrast to traditional extractive techniques, it preserves the integrity of biological specimens, without modifying the nature of the pigments. The spectroscopic apparatus we used is very simple and consists of a common microscope equipped with a monochromator, a photomultiplier, and two pinhole diaphragms. Absorption spectra obtained by means of this apparatus on different species of cyanobacteria are presented.
Key words. Pigments; microscope photometry; absorption spectra; digital microscopy; *Spirulina maxima*; *Spirulina platensis*; *Anabaena azollae*.

The study of spectroscopic pigments and their distribution in cellular structures is usually based on extractive procedures, followed by biochemical or spectroscopic assays. Extractive techniques often present two major drawbacks: they can modify the nature of the components, and they may not be successful in isolating the pigments. Direct spectroscopy on intact specimens has the advantage of preserving the integrity of biological structures or substructures, using a very simple apparatus: an ordinary microscope equipped with a monochromator, two circular pinhole diaphragms and a photomultiplier. This instrument can be controlled by a computer. This article describes absorption spectra of the cyanobacteria *Spirulina maxima*, *Spirulina platensis* and *Anabaena azollae* in order to investigate the distribution of their pigments.

Materials and methods

The apparatus used to perform absorption measurements, which has been previously described in detail[1], consists of a common microscope (Zeiss Standard 16, FRG) equipped with a continuous filter monochromator (Schott, FRG), two circular pinhole diaphragms, and a photomultiplier (Hamamatsu, Japan) connected to a computer (IBM AT, USA). One of the two diaphragms takes the place of the field diaphragm, and the diameter of its image on the object plane is 8 μm. The second diaphragm is placed in the plane of the real and inverted image, and its image on the object plane has a diameter of 0.5 μm. An oil-immersion condenser (n.a. 1.4) and a 100× oil-immersion planapochromatic objective (n.a. 1.25) were used for the measurements. This simple configuration can minimize the typical errors of microspectrophotometry, such as glare, distributional error and photobleaching. The energy striking the samples was $\sim 10^{-9}$ Joule during the 3 min necessary to record the absorption spectrum.

Spirulina maxima and *Spirulina platensis* were grown in seawater supplemented with nitrate, phosphate, bicarbonate and Fe-EDTA[2]. *Anabaena azollae* was obtained by squashing fresh fronds of the water fern *Azolla caroliniana* with a teflon roller and by coarse-filtering the mixture obtained.

Description of spectra, and discussion

In vivo absorption spectra are shown in figures 1–4. Figures 1 and 2 show the spectra of a vegetative cell and of a heterocyst of the symbiotic cyanobacterium, *Anabaena azollae*. The spectrum of the vegetative cell (fig. 1) confirms the presence of the pigments of both photosystem I (chlorophyll a 440, 580, 670 nm, carotenoids 470, 490, 510 nm), and photosystem II (phycoerythrin 500, 550 nm, phycocyanin 620 nm, allophycocyanin 640 nm)[3].

In the heterocyst spectrum (fig. 2) photosystem II is deficient, but still present, contrary to what was previously reported by Donze et al.[4]. Photosystem I is much more

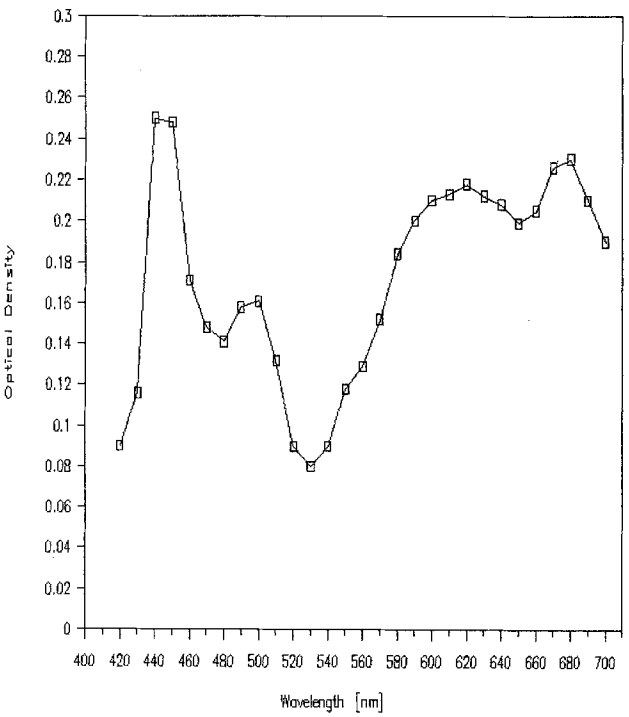

Figure 1. Absorption spectrum of a vegetative cell of the symbiotic cyanobacterium *Anabaena azollae*.

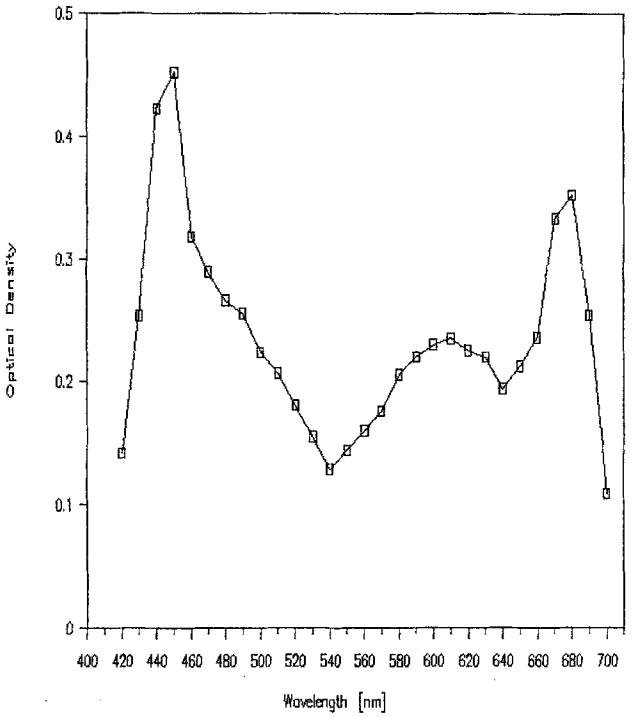

Figure 2. Absorption spectrum of a heterocyst of *Anabaena azollae*.

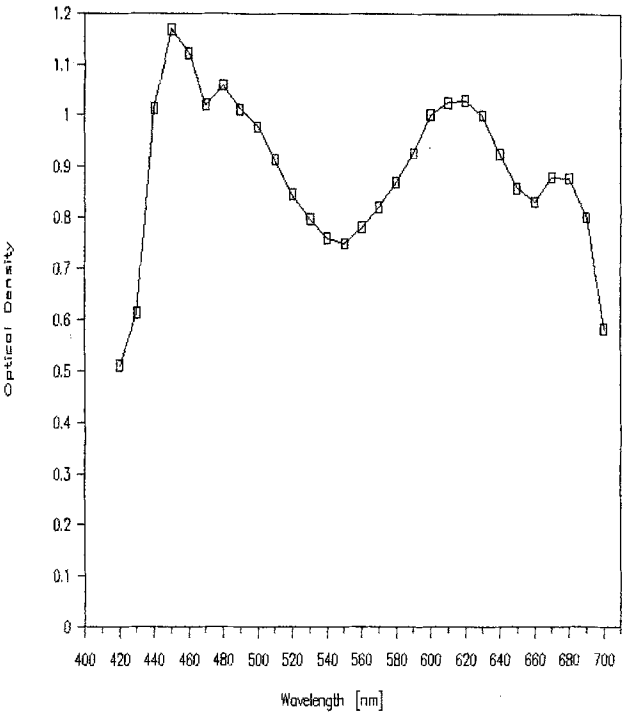

Figure 4. Absorption spectrum of *Spirulina platensis*.

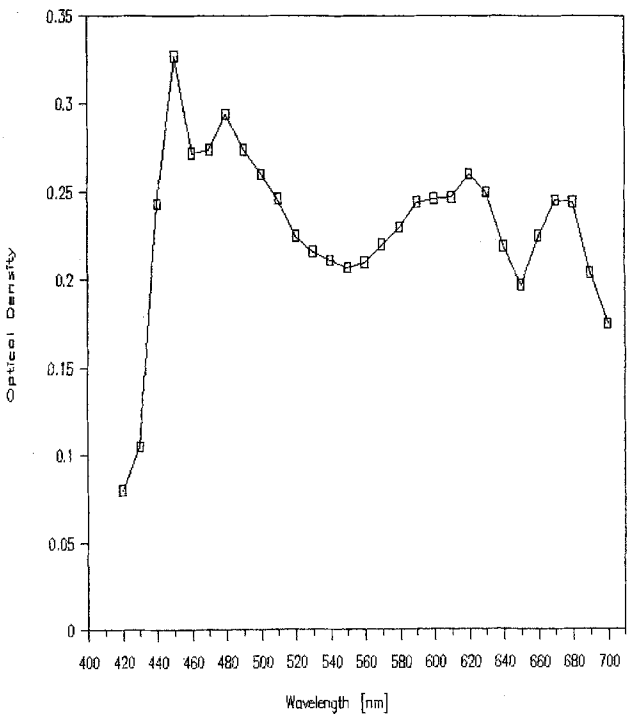

Figure 3. Absorption spectrum of *Spirulina maxima*.

concentrated than in vegetative cells (almost double, as is confirmed by the O.D. value)[5]. Figures 3 and 4 show the absorption spectra of *Spirulina maxima* and *Spirulina platensis*. The trichomes of these non-heterocystous cyanobacteria are composed only of vegetative cells[6], that contain both photosystem I and photosystem II, with the same pigments as the *Anabaena azollae* vegetative cell, but in a different ratio. *Spirulina platensis* spectrum (fig. 4) has a higher optical density value due to the absorption of the out-of-focus cells of its closely coiled trichome.

1 Gualtieri, P., Passarelli, V., and Barsanti, L., J. Photochem. Photobiol., *B3* (1989) 79.
2 Materassi, R., Tredici, M., and Balloni, W., Appl. Microbiol. Biotechnol. *19* (1984) 384.
3 Cohen-Bazire, G., and Bryant, D. A., in: The Biology of Cyanobacteria, pp. 143–190. Eds N. G. Carr and B. A. Whitton. Blackwell, Oxford 1982.
4 Donze, M., Haveman, J., and Schiereck, P., Biochim. biophys. Acta *256* (1972) 157.
5 Wolk, C. P., in: The Biology of Cyanobacteria, pp. 359–386. Eds N. G. Carr and B. A. Whitton. Blackwell, Oxford 1982.
6 Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stainer, R. Y., J. gen. Microbiol. *11* (1979) 1.

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