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In situ photoacoustic spectroscopy of phycobiliproteins in *Gracilaria chilensis*

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Abstract. Phycobiliproteins, the main polypeptidic components of the phycobilisomes (PBS), are biological macromolecules arranged in complex interaction systems to perform light harvesting and conduction. The optical properties of these systems can hardly be studied by conventional spectroscopic techniques. Furthermore this techniques also involve laborious chemical extraction methods. Photoacoustic (PA) spectroscopy was successfully applied to an *in situ* study of the phycobiliproteins expression in the eukaryotic red algae: *Gracilaria chilensis*.

1. INTRODUCTION

Phycobiliproteins are water soluble chromoproteins found in cyanobacteria and eukaryotic red algae. The phycobiliproteins have bilins covalently attached, which are open-chain tetrapyrroles and they absorb the light en the visible region on the spectrum. The phycobiliproteins are organized in subcellular light-harvesting complex called phycobilisomes (PBS). These structures allow to the phycoproteins to be arranged geometrically in a such manner which that optimize the capture of light and transfer of energy. They absorb energy in portions of the visible electromagnetic spectrum that are poorly utilized by chlorophyll. The PBS contain phycobiliproteins which are divided into three classes based on the position of their absorption bands: R-phycoerythrin (R-PE), phycocyanin (PC), and allophycocyanins (APC). The optical absorption maxima for these phycobiliproteins are 566 nm for R-PE, 621 nm for C-PC and 651 nm for APC [1, 2]. The photoacoustic spectroscopy (PAS) technique has been used in a variety of materials [3–7]. In this work photoacoustic spectroscopy (PAS) was applied to investigate the phycobiliproteins expression in an eukaryotic red algae: *Gracilaria chilensis*.

2. MATERIAL AND METHODS

Optical absorption spectra were recorded with a homemade photoacoustic spectrometer. A chopper and monochromator were placed at the output of a 250 W QTH lamp to obtain modulated light at a given wavelength. The samples enclosed in an aluminium PA cell were illuminated within visible range output (400 - 750 [*nm*]). The produced PA signal was detected by a commercial electret microphone and processed by using a preamplifier and a lock-in amplifier. All the spectra were recorded at room temperature with the optical chopper set at 22 Hz. The samples were randomly selected from algae apexes. The apexes of *Gracilaria chilensis* were collected from Pacific Ocean coast at the Coliumo Bay,

in VIII region, Chile. During 4 weeks, the algae apexes were grown in laboratory culture conditions and controlled illumination. Commercially purified phycobiliproteins were not available at the moment; thus, a protein extraction method was utilized to obtain purified PBS and phycobiliproteins also from culture algae apex. The quantification of the extracted amount of phycobiliproteins was calculated from absorbance data [8]. *In situ* PA absorption spectra of the alga and PA transmittance for PBS and phycobiliproteins spectra were also recorded [5].

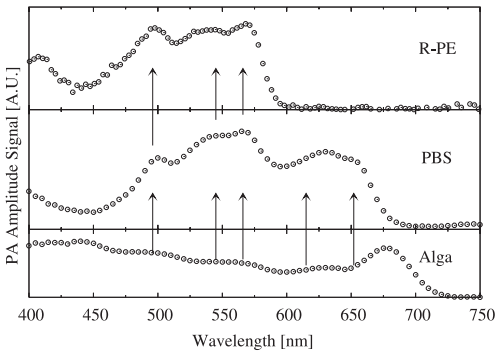

Figure 1. Absorption spectrum of *in situ* alga cuttings (bottom), transmission spectrum of extracted PBS (middle), transmission spectrum of extracted R-PE (top).

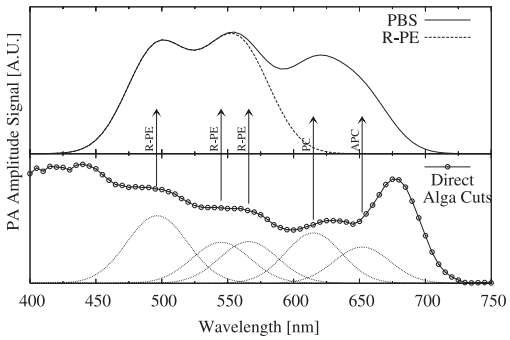

Figure 2. PA spectrum analyzed on Gaussian components: Spectral fitting by Gaussian Peak Deconvolution (bottom). PBS spectra after background substraction and R-PE resolved spectra.

3. RESULTS

The PA spectroscopic measurements of algae cultures are shown in figure 1. The *in situ* optical absorption spectra of the red alga is characterized by a high absorption in the blue (400-500 [*nm*]) and in the red (600-700 [*nm*]) spectral region, caused by a light-harvesting complex containing chlorophyll and carotenoids. It is apparent that the light absorption is increased in the green - yellow spectral region (500-600 [*nm*]) due to the presence of PBS. PBS absorption has a strong band, related to PC and APC but mainly to PE that absorbs green light. The PA spectra do not differ strongly from the spectroscopic results [1, 2]. The PBS and phycobiliproteins are directly identified by its absorption spectra. These results show the influence of the algae structure and its optical properties on the PA signal. The spectroscopic bands of PBS overlap with other optically active bands, such us chlorophyll and carotenoids. It is obvious that it is quite difficult distinguish single or even groups of phycobiliproteins. From "raw" alga PA spectrum, spectral fitting was done. It was possible to solve the band overlapping using gaussian peak deconvolution, after carotenoids and chlorophyll band subtraction. Figure 2 shows an example of sample analyzed on Gaussian components [6, 7]. A number of peaks in different combinations fill the spectral range from 400 to 750 nm. Thus the PBS spectrum can be decompose conveniently in phycobiliproteins groups according to specific bands. The same band structure is resolved despite small differences in relative heights. Quantitative assessment of phycobiliproteins were achieved by optical spectroscopy. The maximum absorption bands are useful as an attempt to quantify the protein expression. The relative amplitude ratio at 620 nm was calculated [9] (Table 1).

Table 1. Expression of Phycobiliproteins inspected by Photoacoustic Spectroscopy

	Phycobiliproteins (Max. Abs. in [nm])		
	R-PE (566)	C-PC (621)	APC (651)
Concentration $\left[\frac{mg}{mL}\right]$	0.03	0.02	0.05
Absorbance	0.44	0.37	0.35
Relative absorbance ratio at 620 nm	1.2	1.0	0.94
PAS signal amplitude	0.0504	0.0429	0.0438
Relative PAS signal amplitude ratio at 620 nm	1.17	1.00	1.02
Gaussian peak height	0.029	0.035	0.0252
Relative Gaussian peak height ratio at 620 nm	0.83	1.00	0.72

4. DISCUSSION

In spite of the diversity and complexity of the red algae pigment composition the three main groups can be photoacoustically defined in accordance with conventional techniques [2]. The key problem for optical methods is still the data quantification. However, this type of study does not aim to determine absolute value but only relative values as function of the wavelength. Observation of bands is very helpful as they show directly the relative expression of the phycobiliproteins in the algae cultures. PA methods are more variable and less sensitive compared to fluorimetric techniques. Continuous cultures can combine the effects of light and nutrient limitation which complicate the interpretation of data acquired *in situ*. PAS can provide *in situ* insights into algal pigment adaptation studies and validate photoacclimation models, in contrast with the time consuming protein extraction methods, fluorimetric and optical spectroscopic techniques.

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