Effect of Pressure on the Absorption Spectra of Phycobiliprotein and *Porphyridium cruentum*

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Changes in the absorption spectra of *P. cruentum* and phycobiliprotein are observed as a function of hydrostatic pressure. With phycobiliprotein both in solution and in algae, increasing hydrostatic pressure results in bathochromic shifts of the absorption maxima. In addition, there is a splitting of the absorption band of phycoerythrin.

When *P. cruentum* is subjected to 250 bars there are increases in absorption by chlorophyll (at 700 and 698 nm), phycocyanin (at 640 nm) and phycoerythrin (at 580 and 550 nm). When a cell free solution of phycobiliprotein is subjected to 300 bars there are increases in absorption by phycoerythrin (at 570 and 550 nm) and phycocyanin (at 634 nm).

Introduction

Pressure is an important parameter in the study of membrane phenomena. It has been useful in discriminating between conformational changes in proteins and in lipid transitions [1]. Thermotropic lipid transitions are more sensitive to pressure than conformational changes in proteins. The present work is a brief survey of the effects of pressure on the spectral properties of phycobiliproteins in cell free solution and in *P. cruentum.*

The application of pressure, instead of elevated temperature to induce spectral changes [2], avoids the possibilities of oxidation reactions, thermal inactivation and decompositions. With solutions of phycobiliprotein there is no possibility of changes in either membrane architecture or state of the membrane lipid contributing to the pressure induced change in spectral properties. The effects of pressure on macromolecules is well known. It enters via the term for the molar free volume change, so that molecular conformations with smaller volume are favored as pressure is increased (*e.g.*, [3]). The molecular volume is smaller when the interatomic interactions are van der Waals forces and hydrogen bonds compared to ionic and hydrophobic bonds (*e.g.*, [4]).

Materials and Methods

A Cary spectrophotometer (model 14R) was used to measure absorption and difference spectra. The absorbance (OD) of the red absorption band of the sample was about 1.5. Difference spectra were measured with the spectrophotometer set for a full scale absorbance of 0.1. Spectral changes were determined by measuring the difference in absorption spectrum between two identical preparations.

The high pressure absorption cell and hydraulic system were constructed by Professor R. Murphy, Dept. of Chemistry, at New York University. The reference preparation was maintained at atmospheric pressure. The sample preparation was subjected to increasing hydrostatic pressure. The difference in absorption ΔOD at wavelength λ, between the reference sample (at zero applied pressure), OD_0 and the sample subjected to a pressure B (in bars) is $\Delta OD = OD(B) - OD_0$. The sensitivity of a substance to a change in pressure (ΔB) is shown as $(\Delta OD/OD)/\Delta B$, where OD is the total absorbance at wavelength at λ.

P. cruentum was cultured as described previously [5]. Phycobiliproteins were extracted from *P. cruentum* by grinding the cells with sand in buffer. The cell debris was removed by centrifugation. The phycobiliprotein in the supernatant was partially purified by fractional precipitation, using ammonium sulfate. The phycobiliprotein precipitate was dialyzed against phosphate buffer pH 7.0, then used for an experiment.

Results and Discussion

Phycobiliproteins: Changes in the absorption spectrum of a cell free solution of phycobiliproteins are measured as a function of hydrostatic pressure.

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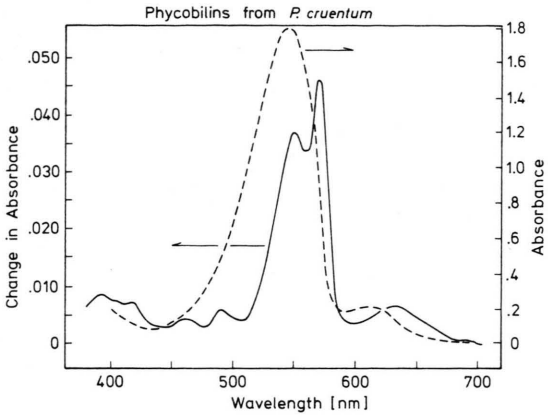

Fig. 1. Difference in absorption spectrum between a sample of phycobiliprotein at a hydrostatic pressure of 300 bars and one at zero applied pressure (curve with solid line). For comparison purposes the absorption spectrum of the phycobiliprotein solution is shown by a curve with short dashed (absorbance is given by scale on right). The phycobiliprotein is a partially purified aqueous extract of *P. cruentum*.

The change in absorption spectrum at 300 bars is shown in Fig. 1. For comparison purposes the absorption spetrum of the phycobilin solution is also shown in Fig. 1; there are broad absorption maxima at 620 and 545 nm, corresponding to phycocyanin and phycoerythrin, respectively. At 300 bars the phycocyanin band gives rise to a red shifted band at 634 nm. The phycoerythrin band gives rise to two bands with maxima at 570 and 550 nm.

The change in absorbance at 660, 570 and 550 nm were measured as a function of pressure. At 660 there is essentially no change in absorption. At 570 and 550 nm there are approximately linear increases in absorbance. The changes, $(\Delta OD/OD)/\Delta B$ are 1.4×10^{-4} bar^{-1} at 570 nm, and 0.61×10^{-4} bar^{-1} at 550 nm.

Intact Organism: Changes in absorption spectrum of *P. cruentum* are measured as a function of hydrostatic pressure. At 250 bars the spectral changes are of the order of 1%. The change in absorption spectrum at 250 bars is shown in Fig. 2. There appears to be a bathochromatic shift of the red absorption band resulting in absorption at longer wavelengths. The difference spectrum shows a 10 nm shift in the red absorption maximum. There also appears to be a shoulder at about 700 nm. The phycocyanin band shifts 5 nm to longer wavelengths. The phycoerythrin gives rise to bands at 550 and 580 nm. The latter represents a 10 to 12 nm red shift. The carotenoid band shifts about 10 nm. A decrease in absorption at 490 nm and increase in absorption at 508 nm indicated a red shift of the carotene absorption band. The blue band of chlorophyll is also shifted about 10 nm to longer wavelengths.

Absorbances increase rather linearly with increasing pressure. At 700 nm the change, $(\Delta OD/OD)/\Delta B$ is 0.6×10^{-4} bar^{-1}, at 550 nm the change is 0.4×10^{-4} bar^{-1}.

If hydrostatic pressure resulted in a small increase in the concentration or optical path length of the high pressure cell, an increase in absorption would result. The difference spectrum, however would be the same as the absorption spectrum of the solution. Since the difference spectrum is quite different from the absorption spectrum of phycobiliprotein or algae, the spectral changes shown in Figs. 1 and 2 cannot be ascribed to a pressure induced change in optical path.

The effect of hydrostatic pressure on *P. cruentum* and phycobiliprotein solution, produces similar spectral changes (*i.e.* a bathochromic shift of absorption maxima). Because of the presence of other pigments *in vitro* it could not be determined if there is a splitting of the absorption band of phycoerythrin in *P. cruentum*, as observed in cell free solution (Fig. 1). Varying the temperature of *P. cruentum* results in spectral changes that are qualitatively similar to those produced by pressure [2].

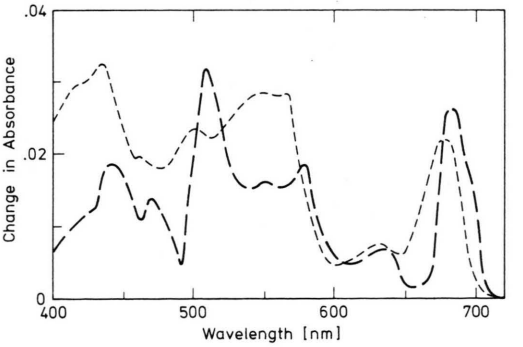

Fig. 2. Difference in absorption spectrum between a suspension of *P. cruentum* at 250 bars and one at zero applied pressure (curve with long dashes). For comparison purposes the absorption spectrum of the algae suspension is shown at a broken line (to obtain absorbance multiply vertical axis by 50).

The spectral changes of the pigment-protein complexes appear to arise from an increase in pigment interaction, resulting from the decrease in molecular volume. It is well known that increased pigment interaction can result in both splitting of the absorption band and a bathochromic shift [6].

It can be argued that the pressure and temperature induced changes in absorption spectra of *P. cruentum* are related to configurational changes in the pigment-protein complex and not a lipid phase change or modification of the skeletal structure.

Acknowledgement

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