Pressure Induced Shifts in Spectral Properties of Pigment-Protein Complexes and Photosynthetic Organisms

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Application of elevated pressure (up to 1200 bars) results in a bathochromic shift of the absorption bands of photosynthetic pigments. The photosynthetic systems studied include: photosystem I particles, chloroplasts, acetone extracts of chloroplasts, AUT particles and light harvesting particles of *Rh. sphaeroides*, light harvesting particles of R 26. Similar spectral changes are observed in all systems. The spectral shifts appear to be associated with pressure-induced changes in the local electric fields of the pigments, rather than changes in the index of refraction of the solvent.

Introduction

Temperature induced shifts in absorption have been reported in a wide variety of photosynthetic organisms, organelles and chlorophyll containing liposomes [1, 2]. High pressure has been shown to induce spectral changes in *Porphyridium cruentum* and phycobiliproteins [3].

Several possibilities have been proposed for the origin of the spectral shift [1, 2] however, the origin of the temperature induced spectral shift is not yet established. Employing high pressure (at constant temperature) to induce the spectral shift eliminates the possibility of changing the population of molecules in the lowest vibrational state.

Materials and Methods

High pressure techniques and equipment used for these measurements are fully described elsewhere [4]. As pressure is increased the liquid phase is compressed to a smaller volume. This results in an increase in absorbance. To correct the absorbance measurements, they are multiplied by the compression of the solvent (*i.e.* V_p/V_1, where V_p is the volume at a pressure of P bars and V_1 is the volume at one bar). The compression data for water and acetone are published by Grindley and Lind [5] and Newitt and Weale [6], respectively. The presence of various concentrations of detergents and salts in the solutions used in the experiments described in this paper may slightly alter the compressibilities.

To determine, if there is a spectral shift and the direction of the spectral shift, the absorbance is measured on each side of the absorption maximum. An increase in absorbance at longer wavelength than the absorption maximum, accompanied by a decrease in absorbance at shorter wavelengths shows a red spectral shift. On the other hand, an increase in absorbance at wavelengths shorter than the absorption maximum, accompanied by a decrease at longer wavelengths shows a blue shift.

Where a linear relationship obtains between applied pressure and absorbance change, the magnitude of the spectral change, ΔOD, is calculated as $M = (\Delta\mathrm{OD}/\mathrm{OD}) \times 10^6/\Delta P$, where ΔP is the pressure difference in bars, and OD is the absorbance. In the figures ΔOD is plotted as a function of pressure.

Chloroplasts were prepared as described by Melis and Duysens [7]. They were suspended in 0.4 M sucrose, 10 mM phosphate buffer, pH 7.8. Photosystem I reaction centers were prepared as described by Mullet *et al.* [8]. They were suspended in 10 mM Tris, pH 7.8 and 0.05% LDAO (lauryl dimethyl amine oxide).

Rhodopseudomonas sphaeroides strain 2.4.1 was cultured as described by Slooten [9]. Chromatophores, light-harvesting pigment-protein complexes, reaction centers, AUT particles, and reaction centers of the carotenoidless mutant R-26 were prepared as described by Romijn [10] (see also Clayton and Clayton [11], Loach *et al.* [12]; Slooten [13]). AUT particles are reaction centers obtained by treating

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chromatophores with 0.3% SDS (sodium dodecyl sulfate). The fraction was purified by gradient centrifugation in the presence of 1 M urea and 0.3% Triton X-100, at pH 10.0. This was followed by dialysis against 0.05 M Tris (pH 8.0) and 0.01 M $MgCl_2$.

Chromatophores were suspended in 10 mM Tris, pH 7.5. Light harvesting pigment-protein complexes were suspended in 50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 0.05% LDAO. AUT particles were suspended in 50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 0.05% LDAO. Reaction centers from R26 were suspended in 10 mM $MgCl_2$, 2 M sucrose, 0.05% LDAO.

Results

1. Acetone extract of chloroplasts

Applying pressure to a system consisting of an acetone extract of chloroplasts results in a bathochromic shift of the chlorophyll absorption band. The absorption maximum of chlorophyll in acetone is at 665 nm. At 670 nm the absorbance increases with pressure ($M = 285\ \mu bar^{-1}$), while at 660 nm the absorbance decreases ($M = -66\ \mu bar^{-1}$) (Fig. 1).

2. Chloroplasts

Increasing the pressure on a suspension of chloroplasts, results in a red shift of the chlorophyll absorption band. The red absorption maximum of chloroplasts is at 676 nm. The shift is shown by an increase in absorbance at 690 nm ($M = 69\ \mu bar^{-1}$), accompanied by a decrease in absorbance at 670 nm ($M = -30\ \mu bar^{-1}$). There is a small decrease in absorbance at 700 nm as pressure is increased.

There is decrease in absorbance at 490 nm but no change at 510 nm. These spectral changes may reflect a red shift of a carotenoid band.

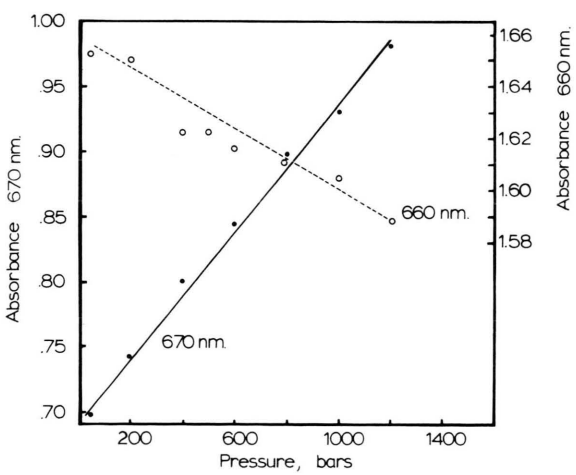

Fig. 1. Change in absorbance of chlorophyll *a* in acetone as a function of pressure, at 25 °C. The absorbance at 670 nm is indicated by closed circles, a solid line and the scale on the left. The absorbance at 660 nm is indicated by open circles, a broken line and the scale on the right.

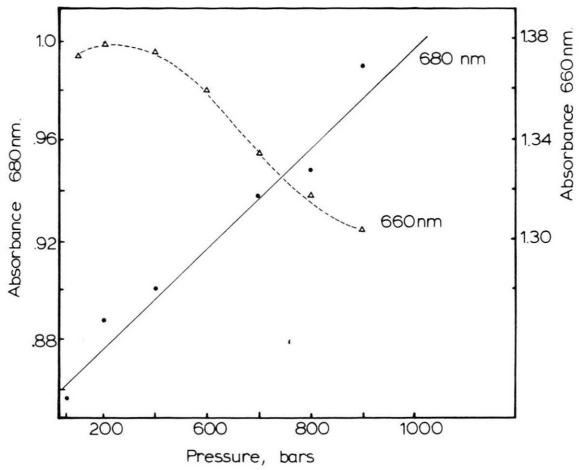

Fig. 2. Change in absorbance of photosystem I reaction centers as a function of pressure, at 25 °C. The absorbance at 680 nm is indicated by solid circles, a solid line and the scale on the right. The absorbance at 660 nm is indicated by open triangles, a broken line and the scale on the left.

3. Photosystem I particles

Increased pressure on photosystem I particles also results in a red shift of the absorption maximum at 667 nm. At 680 nm there is an increase in absorption ($M = 122\ \mu bar^{-1}$), while at 660 nm there is a decrease in absorbance ($M = -54\ \mu bar^{-1}$). At 670 nm the absorbance is constant until about 600 bars, at higher pressure the absorbance decreases (Fig. 2). This behaviour is consistent with the shift in the absorption maximum from 667 nm to longer wavelengths. After passage of the absorption maximum the absorbance decreases on the short wavelength side of the absorption band.

The electrochromic band at about 510 nm also appears to undergo a red shift. At 510 and 500 nm the values of M are 260 and 148 μbar^{-1}, respectively.

The absorption spectrum has a shoulder at 490 nm. This band appears to undergo a red shift

with increasing pressure. The absorbance increases until 600 bars, then remains constant as pressure increases to 1200 bars. This action could indicate the shifting of the absorption maximum toward 490 nm and being in the vicinity of 490 nm from 600 bars to 1200 bars.

The spectral shifts appear larger and clearer in isolated particles (photosystem I) than in intact organisms or intact organelles such as chloroplasts.

4. Whole cells and chromatophores of Rh. spheroides

With chromatophores and whole cells of *Rh. spheroides* increasing pressure results in a general decrease in absorbance, so it was not possible to determine the nature of any spectral shift.

5. AUT particles

In AUT particles the absorption maximum at 805 nm is shifted toward the red as the pressure is increased. The absorbance at 810 nm increases ($M = 81\ \mu bar^{-1}$) and the absorbance at 800 nm decreases ($M = -129\ \mu bar^{-1}$).

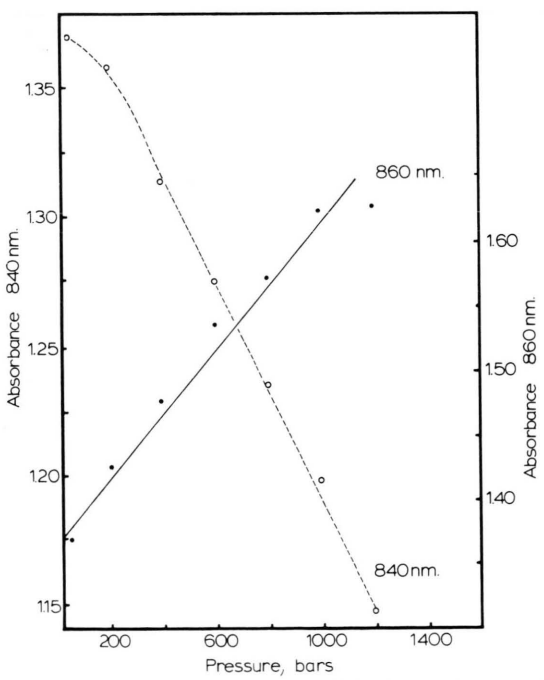

Fig. 3. Change in absorbance of light harvesting particles from *Rhodopseudomonas sphaeroides* as a function of pressure, at 25 °C. The absorbance at 860 nm is indicated by closed circles, a solid line and the scale on the right. The absorbance at 840 nm is indicated by open circles, a broken line and the scale on the left.

6. Light harvesting particles of Rh. spheroides

The spectral changes are particularly large in light harvesting particles of *Rh. spheroides* (Fig. 3). The absorption maximum at 850 nm shifts toward the red as pressure is increased. At 860 nm the value of $M = 192\ \mu bar^{-1}$; at 840 nm $M = -148\ \mu bar^{-1}$. The movement of the maximum at 800 nm is less clear. There is a general decrease in absorbance. At 810 nm $M = -63\ \mu bar^{-1}$; at 790 nm $M = -97\ \mu bar^{-1}$. Since the decrease at 790 nm is greater than at 810 nm, there appears to be a red spectral shift. The general decrease in absorbance could indicate a change or shift in molecular species. That is, the large increase in absorbance at 860 nm may arise, in part, from the decrease in absorbance or molecular concentration of the species whose absorption band is at 800 nm. It was previously shown in a monomolecular film of bacteriochlorophyll, increasing surface pressure increases the absorbance at 896 nm while the absorbance at 794 nm decreases [14].

7. Light harvesting particles of R 26

In light harvesting particles of R 26 the absorption maximum at 865 and 802 nm appear to undergo a red shift as the pressure is increased. The absorbance at 880 and 810 nm increase slightly, while the absorbance at 850 and 790 nm decrease.

Conclusion

Spectral shifts may originate from a variety of effects, for example a change in index of refraction of the solvent, a change in conformation of a chromoprotein, shift in equilibrium between oligometric forms, or change in population of molecules in the lower vibrational states. Since the change in pressure is isothermal, it is unlikely that there is any significant change in population of molecules in the various vibrational states.

The small decrease in absorbance at 700 nm observed with chloroplasts might reflect a narrowing of the absorption band arising from a change in the local electric field around the pigment. It is unlikely that there is a pressure induced redistribution of the molecular population from oligomeric to monomeric forms. Formation of oligomers of

chlorophyll from monomeric forms (in solution) results in a red shift of the absorption maximum [15, 16].

Increased pressure can decrease the molecular volume resulting in tighter packing of pigment-protein complex and solvent. The tighter packing, as well as the change in chromoprotein conformation, can result in an increase in the local electric field.

To distinguish between a spectral shift that may be attributed either to an increase in index of refraction or to an increase in local electric field [17] requires a quantitative analysis of the pressure induced spectral change.

Increasing pressure may, increase the interaction between pigment and the solvent or protein environment, and decrease the pigment-protein volume. The result of this increase in interaction is a bathochromic spectral shift. The increase in molecular packing (or density) can give rise to an increase in local electric field of the pigment.

The increase in solvent density, with increasing pressure, also results in an increase in the refractive index of the solvent. An increase in refractive index is expected to cause a red shift of the spectrum [18].

The quadratic Stark effect, which results in slight spectral shifts, is proportional to the square of the electric field strength. To determine if the spectral shift may be the result of the increase in electric field, then the spectral shift should be proportional to $\varrho^{4/3}$, where ϱ is density. The distance between solvent moleculs is proportional to the reciprocal of the cube root of the density; the electric field of charged particles is proportional to the reciprocal of the square of the distance between charges.

If the spectral shift is attributed to a pressure-induced change in refractive index, then the spectral shift is roughly proportional to $(\varrho^2 - 1)/(2\varrho^2 + 1)$, where solvent density is taken as proportional to refractive index [18, 19]. Whether the spectral shift is plotted as a function of this expression or as the 4/3 power of density, there is no significant difference. In addition, it appears that the pressure range used in this study is insufficient to induce a change in refractive index which might account for the observed spectral shifts.

Peak shifts may also be related to a model which includes, changes in volume of the system, the force constants of the excited and ground states, and coupling of the force constant to the bulk modulus of the medium [20]. The pressure range in our study, however, is too small to determine if this model is applicable to our system.

In conclusion, the pressure-induced spectral shifts do not seem to arise from an index of refraction (or solvent) effect or a quadratic Stark effect. Since protein-free preparations of chlorophyll (in acetone) also show pressure-induced spectral shifts, it is apparent that neither protein conformational changes nor pigment-protein interactions are required (for the spectral shifts). Similar spectral changes (with only minor differences) are observed in all the systems examined. The one common component in all the systems is the chromophore (chlorophyll or bacterio-chlorophyll). It appears that the spectral shifts arise from pressure-induced changes in the local electric field of the pigment (*e.g.* those associated with pigment-pigment or pigment-solvent interactions).

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