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Evidence for high-pressure-induced rupture of hydrogen bonds in LH2 photosynthetic antenna pigment-protein complexes

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Abstract. The bacteriochlorophyll *a*-containing LH2 light harvesting complex is an integral membrane protein that catalyzes the photosynthetic process in purple photosynthetic bacteria. The LH2 complexes from *Rhodobacter sphaeroides* show characteristic strong absorbance at 800 and 850 nm due to the bacteriochlorophyll *a* molecules confined in two separate areas of the protein. Using these cofactors as intrinsic probes to monitor changes in membrane protein structure, we investigate the response to high hydrostatic pressure up to 2.1 GPa of LH2 complexes embedded into natural membrane environment or extracted with detergent. We demonstrate that high pressure does induce significant alterations to the tertiary structure of the protein in proximity of the protein-bound bacteriochlorophyll *a* molecules, including breakage of the hydrogen bond they are involved in. The membrane-embedded complexes appear more resilient to damaging effects of the compression than the complexes extracted into detergent environment. This difference has tentatively been explained by more compact structure of the membrane-embedded complexes.

1. Introduction

Membrane proteins are ubiquitous in life-supporting processes, such as signal transmission, immune response, photosynthesis, and respiration, to name but a few (see [1] for a review). The peripheral antenna of purple photosynthetic bacteria, or light-harvesting complex 2 (LH2), is one of the best-characterized membrane proteins. The crystal structure of isolated LH2 (from *Rhodopseudomonas (Rps.) acidophila* strain 10050) [2] reveals a highly symmetric ring of nine pigment-protein subunits, each containing two (α and β) helical membrane-spanning polypeptides, three non-covalently bound bacteriochlorophyll *a* (Bchl) molecules, and a carotenoid pigment. The α- and β-polypeptides form two concentric cylinders providing, respectively, inside and outside support to the cofactor rings between them (see figure 1). A ring of 18 tightly coupled Bchl cofactors in a waterwheel-like arrangement are seen in the lumenal part of the photosynthetic membrane (bottom side of figure 1). It is responsible for the intense near infrared absorption of the LH2 complex at about 850 nm (thus the name, B850). The position of the B850 cofactors relative to each other is determined by hydrogen bonds to the surrounding protein as well as by coordinative bonds with the central magnesium ion of the Bchls. Another ring of 9 Bchl molecules is located towards the polar cytoplasmic part of the membrane (top side of figure 1), being in charge with the absorption band at 800 nm (B800). Both the B850 and B800 bands are related to the Q_y electronic transition in the component Bchl molecules.

High-pressure modulation of structure and function of proteins is an established technique (see [3] for a review). Providing fundamental insights about phase properties, high-pressure measurements can contribute into understanding of various elements that help to stabilize, purify, and storage the membrane proteins. Only few high-pressure studies have so far concerned the LH2 antenna complexes [4-7]. These early works, which generally were limited to 0.6 GPa, have shown that increasing the pressure at physiological temperatures causes substantial red shift and broadening of the Bchl absorption bands. A loss of the 800 nm-absorbing Bchls in the purified LH2 complexes from *Rhodobacter (Rb.) sphaeroides* 2.4.1 and *Rps. acidophila* 10050 suggested pressure-induced alterations to the tertiary structure of the protein in proximity to the membrane/cytosol interface [6].

However, no significant perturbation of the oligomerization state of the polypeptides, distortion of the protein-bound B850 Bchl molecules, or breakage of the hydrogen bond they are involved in was observed.

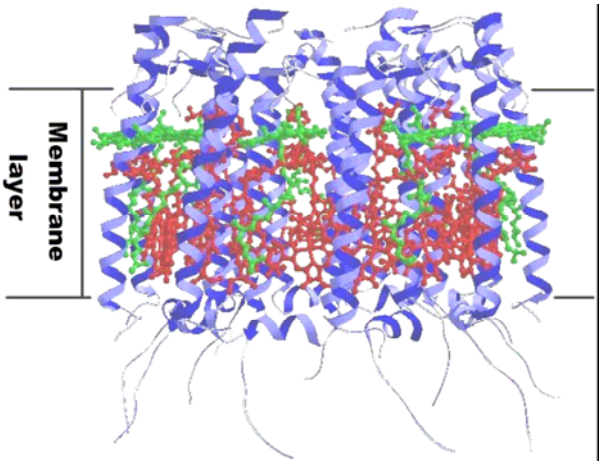

Figure 1. Structural view of the LH2 pigment-protein complex. For clarity, only the B850 (red) and B800 (green) Bchl pigment cofactors and transmembrane α- helices are shown. The lipid bilayer covering hydrophobic parts of the complex is also schematically presented.

That the oligomeric protein does not dissociate at pressures as high as 0.6 GPa is surprising. From literature, it is known that most of the multi-chain soluble proteins unfold at room temperature already below 0.2 GPa [8]. This process is driven by a decrease in volume, which results from both the release of intra-molecular voids and the expose of the interior of the protein to solvent. Thus motivated, we make a step forward and investigate a spectral response of the LH2 complex to unusually high for the proteins hydrostatic pressures up to 2.1 GPa. A comparison is made between the electronic absorption spectra of Bchl pigments in LH2 complexes from *Rb. sphaeroides* either isolated or embedded in the native membranes.

2. Experimental

Membrane vesicles (chromatophores) of genetically engineered *Rb. sphaeroides* bacteria in which only the LH2 antenna protein is synthesized were prepared as described earlier [9]. The protein stocks were stored at liquid nitrogen temperature and thawed before using. The samples were diluted with a buffer containing 20 mM Tris, 1 mM EDTA, 0.1 M NaCl (pH 8.0) to obtain an optical density of about 0.4 at the B850 absorption band maximum in the assembled high-pressure cell. As shown in [10], the pH of the Tris buffer is almost pressure insensitive. About 1% (w/w) lauryldimethylaminoxide (LDAO) in the solution with the purified LH2 complexes was used to ensure well-isolated complexes in detergent micelles [9]. No degradation of the samples was observed within 23 hours at ambient pressure and temperature.

A micro-spectroscopy setup was arranged around a Zeiss Axioskop 2 microscope, using a 30 W power-stabilized halogen incandescent lamp as a light source. The light coming through the sample in a diamond anvil cell (DAC, D-02 from Diacell Products Ltd.) was collected by the microscope's objective and focused into a 200 μm diameter glass fiber. The absorption spectra were recorded either with a BWTek BTC111E series CCD spectrometer or a 0.3 m spectrograph (Shamrock SR-303i, Andor), combined with an electrically cooled CCD camera (EEV 30-11, Andor).

The sample solution was injected into a 0.3 mm-diameter hole in about 0.35-mm thick stainless steel gasket of the DAC. After the DAC closure the pressure was increased with an average rate of 15-150 MPa per minute in different experiments. The pressure in DAC was determined following a shift of a 690.3 nm emission line of a Sm^{2+}-doped SrFCl micro-crystalline pressure sensor mounted into the

sample volume. The pressure sensitivity of 1.1 nm/GPa of this sensor is linear over a broad pressure range [11]. The luminescence of the sensor crystal was recorded by a 0.85 m SPEX 1402 spectrometer. The absorption spectra measured at different pressures were corrected for the background absorbance of the DAC, filled with only the buffer solution and the detergent.

3. Results and discussion

3.1. Absorption spectra of LH2 complexes at ambient pressure

Figure 2A compares the absorption spectra of isolated and membrane-soaked LH2 complexes of *Rb. sphaeroides* measured at ambient pressure and temperature over the spectral range of 550 – 1000 nm. As seen, the spectra of both samples, revealing two intense, well-separated B800 and B850 bands of expected stoichiometric integral intensity ratio of ~1/2, match perfectly well.

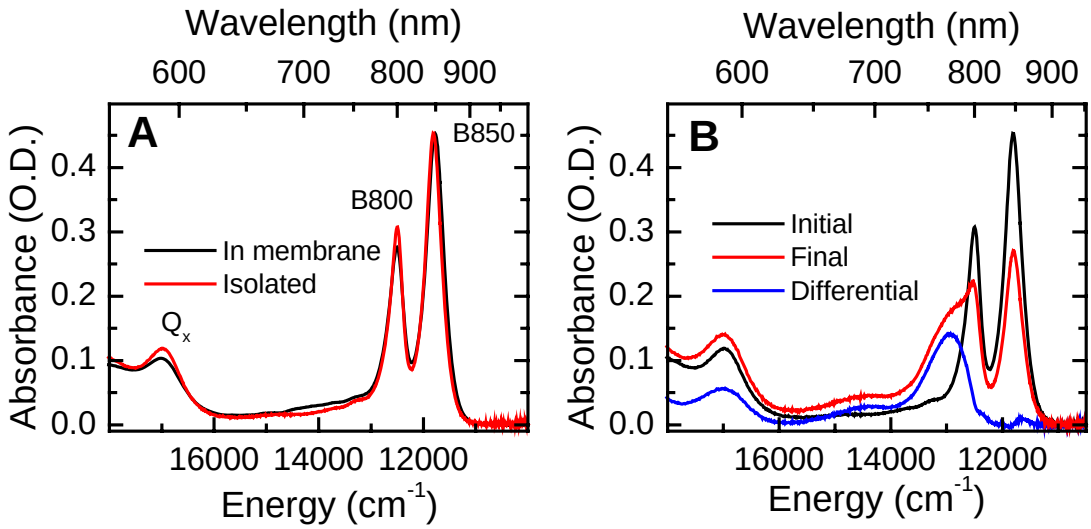

Figure 2. (A) Absorption spectra of membrane-associated and LDAO-isolated LH2 complexes from *Rb. sphaeroides* at ambient pressure. (B) The spectra of LDAO-isolated LH2 complexes recorded at ambient pressure before and after the measurement cycle of figure 3. The difference spectrum (blue curve) was acquired by subtracting the initial spectrum (black curve) from the normalized (by the B850 peak intensity) final spectrum (red curve) under a simplifying assumption that the B800 and B850 bands decay proportionally under pressure.

Figure 2B demonstrates an incomplete recovery of the spectrum of the detergent-isolated complexes after pressure release, subsequent to about 2.5 hours trials of figure 3A where maximum pressure of 2.1 GPa was attained. The difference spectrum that was acquired by subtracting the initial ambient-pressure spectrum from the final spectrum after pressure release shows a maximum at 773.4 nm. The 750-780 nm spectral region is commonly related to non-functional (solubilized) pigments [12], suggesting that the difference spectrum in figure 2B is due to individual Bchl molecules in contact with the buffer-detergent solvent phase. Assuming that the absorbance of the pigments either solubilized or organized into the B850 aggregate is roughly the same, we estimate that in total about 40% of the LH2 complexes in figure 2B have been broken down by the pressure treatment. This is in stark contrast with the membrane-protected particles that largely maintain their intactness throughout the pressure handling (data not shown).

3.2. Pressure-induced variations of the absorption spectra

Pressurizing of the membrane-associated LH2 complexes causes a continuous, quasi-linear shift of the B800 and B850 absorption bands to longer wavelengths (red shift), accompanied with some band broadening. In the low-pressure region where comparison is feasible, the results of the present work are rather similar to the previous data [5,6]. The red shift rate of the B850 band (–626± 40 cm^{-1} GPa^{-1}) more than three times surpasses that of the B800 band (-204 ± 23 cm^{-1} GPa^{-1}). According to figure 3B there is considerable slow-down of the shift rate of both the B800 and B850 bands at ~0.6 GPa.

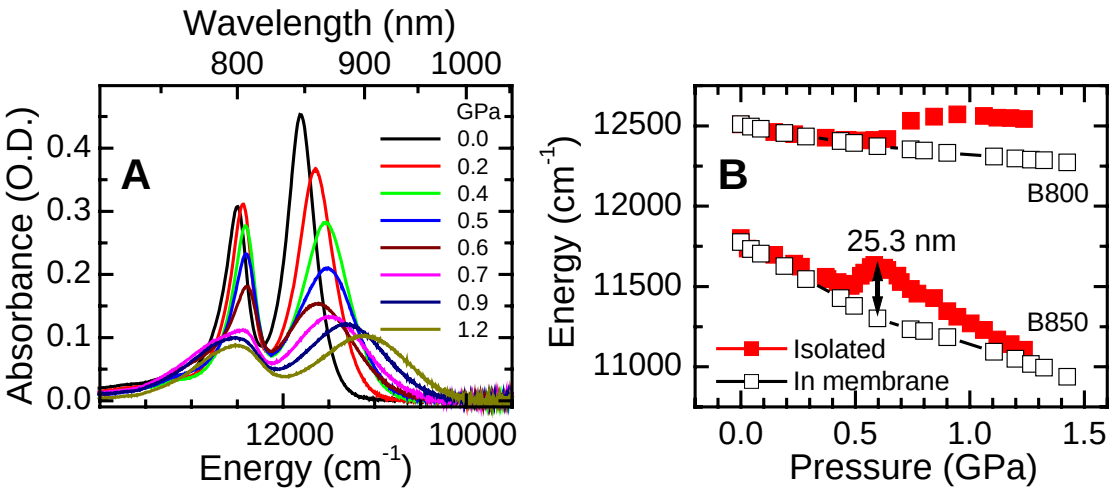

Figure 3. (A) Pressure dependence of the absorption spectra of LDAO-isolated LH2 complexes from *Rb. sphaeroides*. (B) Absorption band positions for the membrane-associated (open rings) and the purified isolated (filled squares) LH2 complexes as a function of pressure.

For the detergent-isolated LH2 complexes, the pressure dependence of the absorption spectra is shown on figure 3A. At low pressures up to ~0.5 GPa the spectra behave similar to the ones of the membrane-associated complexes. However, toward higher pressures striking, previously unobserved, differences appear. Firstly, the usual red shift of the B850 band is abruptly, between 0.5 and 0.6 GPa, replaced by a considerable blue shift that in its maximum deviation from the absorption peak position of the membrane samples reaches 332 cm^{-1} or 25.3 nm. Past ~0.6 GPa the blue shift of the B850 band is yet again overtaken by a red shift. The spectra of the isolated and membrane-associated complexes approach each other once more at pressures above ~1.3 GPa. Secondly, as already mentioned, a partial breakdown of purified complexes takes place during the pressure treatment. In figure 3A, this is demonstrated by simultaneous decrease of both the B800 and B850 bands and concomitant increase of the short-wavelength shoulder of the B800 band. The pressure damage intensifies past ~0.7 GPa, as evidenced by the strongly deviated courses of the B800 band in purified and membrane complexes (figure 3B). The damage is permanent, so that the final spectrum measured after passing the whole pressure cycle is rather unlike the spectrum before squeezing (see figure 2B).

A blue shift at ambient pressure of the B850 absorption band was previously observed for modified LH2 complexes of *Rb. sphaeroides* [13]. By site-directed mutagenesis analysis, this shift was correlated with a breakage of both 2-acetyl carbonyl hydrogen bonds that coordinate the two Bchls in the basic unit of the B850 annular to the surrounding protein (see Introduction). It was also demonstrated that the changed hydrogen bonding pattern leads to ~26 nm blue shift in the B850 absorbance measured at 77 K. A comparison with the present 25.3 nm pressure-induced shift in figure 3B strongly implies that essentially all hydrogen bonds to the B850 molecules are broken by external high hydrostatic pressure.

4. Concluding remarks

The lipid bilayer membrane is a natural background for the LH2 protein and it is quite expected that the membrane-embedded complexes appear more resilient to the damaging effects of compression by external hydrostatic pressure than the complexes extracted into the fatty detergent environment. For the well-isolated antenna complexes we have clearly demonstrated that high pressure does induce significant distortion not only of the binding site of the protein-bound 800 nm-absorbing Bchl molecules as found earlier [6], but also of the 850 nm-absorbing Bchls, including rapture of the hydrogen bonds they are involved in. While in the membranes the pressure-induced alterations to the tertiary structure of the LH2 protein seem to concentrate in proximity of the membrane/cytosol interface at the N-terminal side, in isolated species the whole bulk of the protein is involved.

A change of the pressure-induced absorption band shift rate for the membrane-associated LH2 complexes around 0.6 GPa (figure 3B) implies significant decrease of the compressibility of the samples at higher pressures. The dependences for isolated and membrane complexes, being close below 0.5 GPa, approach each other again at pressures around 1.3 GPa. It is thus quite probable that also in the membranes the pressure-induced distortions of the hydrogen bond pattern take place that, however, are more gradual and less shattering than observed for the isolated complexes.

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