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High pressure near infrared study of the mutated light-harvesting complex LH2

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Abstract

The pressure sensitivities of the near infrared spectra of the light-harvesting (LH2) complex and a mutant complex with a simplified BChl-B850 binding pocket were compared. In the mutant an abrupt change in the spectral properties occurred at 250 MPa, which was not observed with the native sample. Increased disorder due to collapse of the chromophore pocket is suggested.

Key words
- Light-harvesting complex
- High pressure optical spectroscopy
- Protein compressibility
- Antenna proteins
- High pressure light scattering

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Introduction

The peripheral light-harvesting (LH2) complex from *Rhodobacter sphaeroides* (*Rb. sphaeroides*) constitutes an ideal model to investigate the effect of external stress on the stability of membrane proteins. The LH2 complex contains several pigment molecules, which constitute a series of intrinsic molecular probes. These individual chromophores, therefore, are able to monitor localized structural changes when examined by spectroscopic methods as a function of external stresses such as applied hydrostatic pressure. Protein-cavities or voids, if they are empty in the interior of soluble proteins, may result in poor packing. The correlation between protein stability and packing quality, however, is disputed (1). Internal voids can be induced in membrane embedded LH2 or related pigment-binding proteins by either the removal of a specific chromophore (2) or as a result of site-directed mutagenesis (3).

The peripheral antenna of purple photosynthetic bacteria is one of the best-characterized membrane proteins. These proteins, which are present in many photosynthetic bacteria, absorb solar photons and transfer the resulting excitonic energy to the so-called core antenna protein (or LH1) that surrounds the bacterial reaction centers. From the LH1, the energy is further transferred to the reaction centers, where it is transformed into potential chemical energy. This photosynthetic machinery is embedded in the thylakoid membrane, in bacteria typically in the intra-cytoplasmatic membrane. In the case of *Rb. sphaeroides*, these membranes are spherical invaginations of the intra-cytoplasmatic membrane, which become vesicles upon cell disruption (Figure 1).

X-ray crystallography studies of the LH2 from *Rhodopseudomonas* (*Rps.*) *acidophilia* 10050 have revealed the structural organization of this pigment-protein complex (4) (Figure 2A). This LH2 has a nonameric ring structure. In these nonamers, each unit contains two small single membrane-spanning

polypeptides called α and ß. The nine α-apo-proteins form a hollow cylinder with the nine ß-apo-proteins arranged radially outside. One (or two) carotenoid (rhodopsin glucoside) and three bacteriochlorophyll *a* (BChl *a*) molecules are non-covalently attached to each α/ß pair. Most of the α-polypeptide/ß-polypeptide interactions in LH2 occur close to the membrane/water interfaces, between the C- and N-membrane-hugging termini, while the pigments (both BChl and carotenoid molecules) mediate most of the α-ß contacts in the hydrophobic phase. In LH2, the 27 BChl molecules are organized into two discrete pools. Eighteen of the BChl molecules are sandwiched between the α- and ß-apo-proteins and form a ring of overlapping BChl in close contact (see Figure 2A). These molecules absorb in the near-infrared at 850 nm and are known as the BChl-BChl-B850 molecules. The other nine BChl molecules lie toward the cytoplasmic side of the membrane. These pigments exhibit spectroscopic properties close to monomeric BChls. They absorb in the near-infrared at 800 nm, and are denoted BChl-BChl-B800. Free in organic solvents, the Q_y electronic transition of monomeric BChl *a* is located at 770 nm. The physicochemical mechanisms underlying the shift of this transition to 800 or 850 nm when these molecules are bound to LH2 proteins have been extensively studied. For BChl-BChl-B800, the mechanism essentially depends on the interactions between the BChl molecules and their specific microenvironment (5), while for BChl-BChl-B850 it results from a combination between BChl/BChl interactions and interactions between each BChl and the surrounding protein (6-8). These interactions are very sensitive to localized structural (environmental) changes and therefore the positions of the Q_y transitions constitute intrinsic molecular probes that are able to monitor the integrity of the LH2 structure. The electronic properties of LH2 yield information both about the structure of the BChl binding sites within this protein (which in turns yields information about the tertiary structure of the polypeptides in the vicinity of these molecules) and indirectly (although in a highly persuasive manner) about its quaternary structure. As a result, the LH2 family of proteins is ideal for the study of the stability of membrane-embedded hydrophobic α-helical membrane helices.

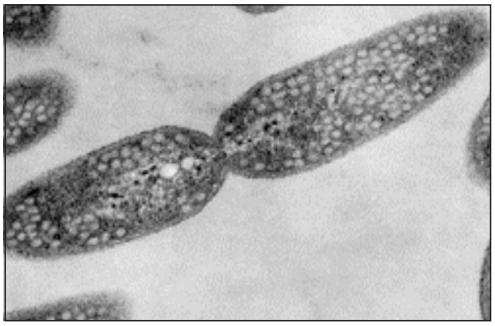

Figure 1. *Rhodobacter sphaeroides* cells during division with spherical membrane invaginations, which contain LH2 complexes (cell size: 20 nm).

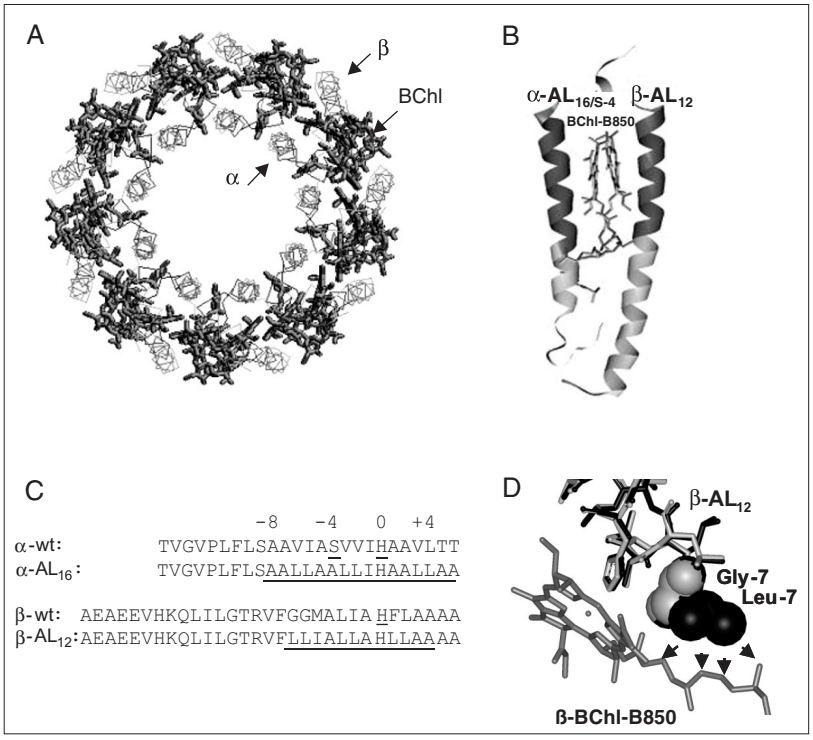

Figure 2. *A*, Schematic view of the LH2 complex from the plane of the membrane. *B*, Elementary α and ß subunits with BChl-B850 (the mutated regions are shown in grey). *C*, Amino acid sequences of LH2 apoproteins and mutants. *D*, Structural alignment of residues at position-7 of wild-type LH2-ß (grey) and mutant LH2-ß (black). BChl = bacteriochlorophyll; wt = wild type; AL = alanine mutant.

Previously, we have mutated both the α- and ß-apo-proteins of LH2 to produce complexes with substantially simplified BChl-B850 binding pockets (see Figure 2B,C). In spite of the extensive mutations, the spectral properties of these complexes remain unchanged (9,10). However, the mutations induced local packing defects around the BChl-B850 molecules (Figure 2D) and a significant reduction in thermal stability. Various antenna pigment-protein complexes from purple photosynthetic bacteria have been studied at high pressure in detergent, both at ambient temperature (2,11-13) and at cryogenic temperatures (14). These studies have shown that increasing the pressure leads to a substantial red shift and broadening of the long wavelength Q_y absorption band both in LH1 and in LH2 in detergent. In the present study, the properties of LH2 are compared to the properties of the LH2 mutant complex in which the residues at the BChl/polypeptide interface have been systematically mutated (Figure 2B-D). This permits us to monitor the stability of model membrane proteins under external stress in the native lipid environment.

Material and Methods

Bacterial strains, plasmids, gene transfer, and growth conditions

The bacterial strains used in the present study were *E. coli* strain S17-1 (*thi pro hsdR*$^-$ *hsdM*$^+$ *recA* RP4-2 (Tc::mu Kan::Tn7), *Rb. sphaeroides* strain DD13 (genomic deletion of both *pucBA* and *pufBALMX*; insertion of Sm^R and Kan^R genes, respectively) (15). The mobilizable plasmids used were based on pRKCBC1 (Tc^R, a derivative of pRK415; insertion of a 4.4-kb fragment encompassing *pucBAC*); briefly, this expression vector contains the *pucBA* genes as a 420-base pair *Kpn*I-*Bam*HI insert (15). Growth conditions for *E. coli* and *Rb. sphaeroides* were as described in Ref. 16. Conjugative transfer of plasmid from *E. coli* S17-1 to *Rb. sphaeroides* was performed as described in Ref. 16.

Construction of the LH2 complex with the model BChl-BChl-B850 site was carried out as described in Refs. 9,10. Membranes were prepared from cells grown semi-aerobically in the dark by disruption in a French pressure cell and subsequent centrifugation on a sucrose step gradient (10). Dynamic light scattering experiments on LH2 vesicles under pressure were performed using an ALV goniometer-correlator system (Fa. ALV-Laser GmBH, Langen, Germany) and a frequency doubled NdYag laser (Coherent GmbH, 150 mW). The sample was transferred to a specially designed four-window high-pressure cell manufactured by SITEC (Zürich, Switzerland). Optical absorption spectra were measured using the same cell, which was connected by optical fibers to a Perkin-Elmer UV/Vis Lambda II spectrometer (Überlingen, Germany).

Results

Dynamic light scattering experiments were performed with LH2 vesicles as function of scattering angle at ambient pressure. The intensity correlation function was analyzed in terms of two dynamic components: a fast component contributing 30% to the total scattering intensity and a slow fraction. From the resulting diffusion constants, we derive an average hydrodynamic radius using the Stokes-Einstein relation:

$$D = k_B T/(6 \pi \eta R_H) \qquad \text{(Eq. 1)}$$

where η is the viscosity of the solvent. R_H of the small particles at ambient pressure is 21 ± 2 nm, while the radius of larger particles is 78 ± 2 nm as shown in Figure 3. Since the scattering intensity varies with the 6th power of the radius, and the large particles scatter only twice as much as the small particles, we conclude that the vesicles with an average size of 21 nm outnumber the larger aggre-

gates by a factor of 60,000. The same experiments were performed at a 90º scattering angle in the pressure cell. The scattering intensity due to the two components remained approximately constant with pressure. However, the radius of the large particles decreased with pressure. Their effective compressibility, assuming the shape of the vesicle to be approximately spherical, can be estimated from:

$$3\ \delta R_H/(\delta p\ R_H) \approx \delta V_{ves}/(\delta p\ V_{ves}) = -\beta_T \quad \text{(Eq. 2)}$$

where V_{ves} is the volume of the vesicle and β_T denotes the isothermal compressibility. From the slope in Figure 3, correcting for the increase of the refractive index with pressure, we obtain $\beta_T \approx 0.9\ GPa^{-1}$. This value is almost twice as high as the compressibility of water (0.56 GPa^{-1}). The large apparent compressibility suggests that the shape of the vesicular aggregates becomes more asymmetric under pressure. The radius of the small particles varies only slightly with pressure, consistent with a compressibility dominated by the included water. The light scattering experiment demonstrated that the LH2 vesicles remain stable up to 500 MPa.

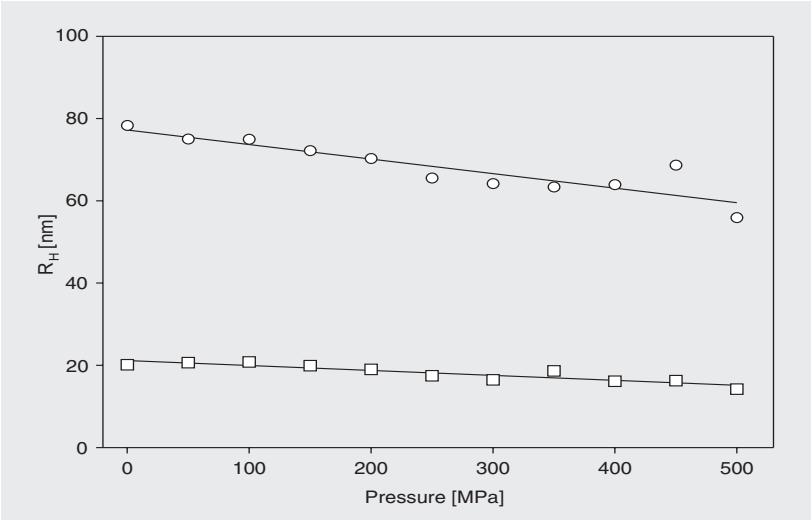

Figure 3. Hydrodynamic radius (R_H) of the LH2 vesicles at 20ºC versus pressure, corrected for solvent viscosity.

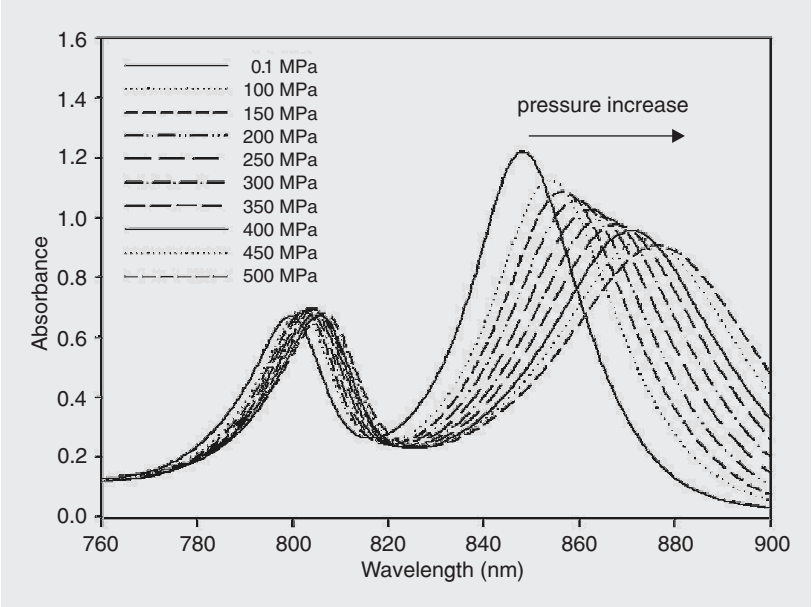

Figure 4. Near infrared absorption spectrum of wild-type LH2 vesicles.

Figure 4 shows the near infrared spectrum of wild-type LH2 (same scale for mutant and wild-type spectrum). The BChl-B850 band is stronger than the BChl-B800 band since 18 versus 9 BChl *a* molecules are contributing to the absorption. As shown previously for LH2 complexes in detergent, the application of pressure led to a significant red shift of the absorption maxima, which was more pronounced for BChl-B850 (11,14). The latter made excitonic contributions reflecting direct interactions of BChl *a* dimers, in contrast to BChl-B800, in which the molecules are well separated and excitonic effects are negligible. The BChl-B800 pressure shift of 0.1 cm^{-1}/MPa is typical for isolated chromophores embedded in proteins and glassy matrices and reflects the protein compressibility (17). The pressure shift of BChl-B850 is about seven times larger. This indicates that the interactions of the BChl *a* dimers increase with pressure, possibly due to reduced distances. The width of the BChl-B800 band remained approximately constant near 9 nm. In contrast, the width of BChl-B850 increased from 12 nm

at ambient pressure to about 19 nm at 500 MPa. Figure 5 shows the BChl-B850 spectrum of the mutant complex. The mutant absorption band was red-shifted with increasing pressure similar to the red shift of this band in the wild type. The increase in line-width, however, was significantly more pronounced in the mutant BChl-B850 band. The line shapes of the absorption bands in the frequency domain were reasonably well approximated by a Gaussian distribution, indicating a dominance of inhomogeneous broadening. Figure 6 compares the pressure shifts of wild-type and mutant BChl-B850. At low pressures up to 200 MPa, the pressure shifts were identical within the experimental error, about -0.75 cm^{-1}/MPa. This suggests that the chromophore coupling in the BChl-B850 ring is similar for wild type and mutant. However, above 200 MPa, the negative pressure shift of the mutant decreased, while it remained constant for the wild type. This result indicates that increasing the pressure beyond 200 MPa does not further enhance the chromophore interactions in the mutant. The change in the pressure sensitivity was quite abrupt, therefore suggesting a structural change. This is supported by the pressure dependence of the bandwidth shown in Figure 7. The pressure sensitivity of the Gaussian width of the BChl-B850 band of wild-type LH2 remained approximately constant with pressure. In contrast, the Gaussian width of the mutant BChl-B850 band abruptly increased at 200 MPa, coinciding with the pressure at which the negative pressure sensitivity of the band decreased. This indicates enhance energetic disorder, possibly related to loss of secondary structure. Inspection of the BChl-B850 absorption spectrum of the mutant in Figure 5 suggests a transition from a reasonably well-defined state absorbing at 850 nm towards a more disordered structural state absorbing near 875 nm. In the latter the chromophore-chromophore interactions have reached a limiting value.

A possible origin of the abrupt spectral changes could be a collapse of the protein pocket surrounding the chromophores, which may be less stable in the mutant than in wild-

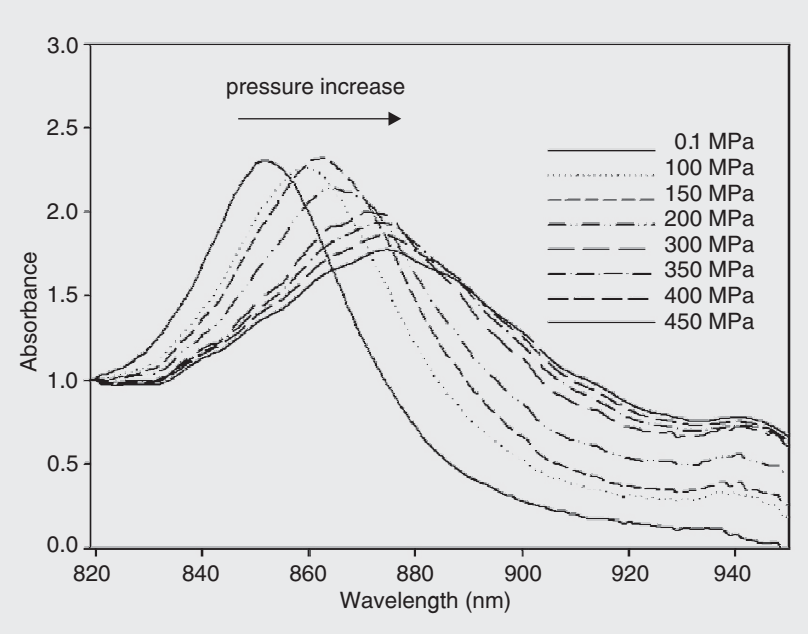

Figure 5. Near infrared absorption spectra of the mutant LH2 complex versus the indicated pressures.

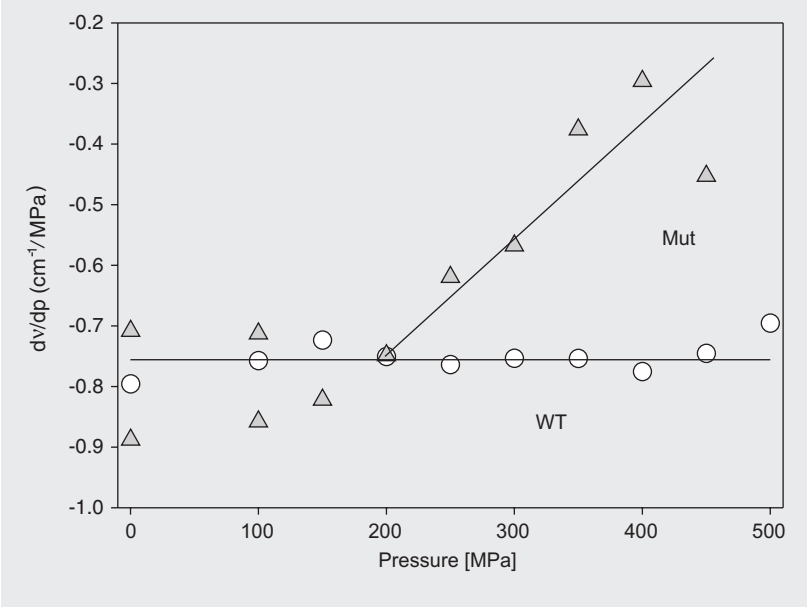

Figure 6. The pressure shift of the BChl-B850 line maximum (dv/dp) of wild-type (WT, circles) and mutant (Mut, triangles) LH2 from Gaussian fits in the frequency domain.

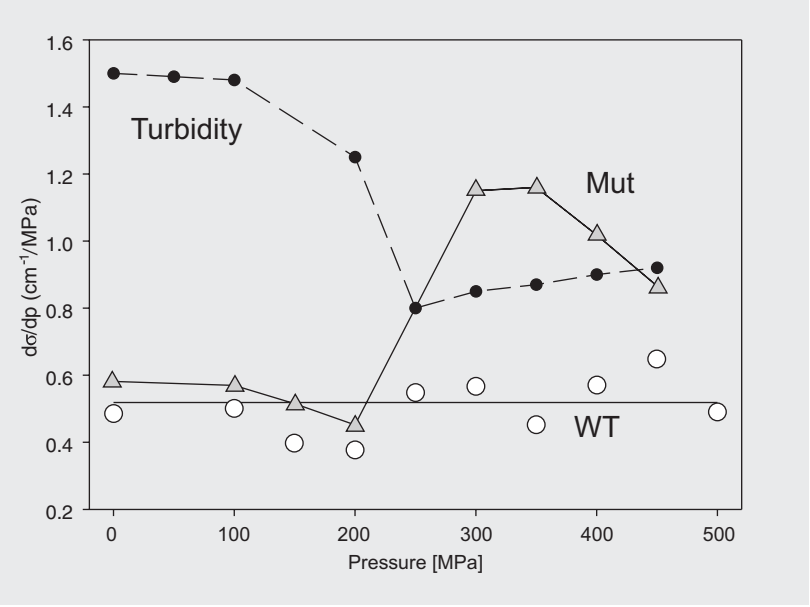

Figure 7. Gaussian line-width of BChl-B850 wild-type (WT, open circles) and mutant (Mut, triangles) and turbidity (filled circles) at 649 nm (OD -1) of the mutant sample.

type LH2. Interestingly, as shown in Figure 7, in the same pressure range we observe an abrupt decrease in the Raleigh scattering intensity for the mutant. This result implies that mutant-LH2 vesicles dissociate at least partially under pressure. This correlation is somewhat surprising since one expects that only the local properties of the protein structure in the vicinity of the chromophore should affect the absorption spectra. Dynamic light scattering experiments performed with the mutant preparation indicate that the number of large vesicle (80 nm) was significantly enhanced compared to the native preparation. That structural changes of the vesicles and of the membrane can modify the pressure sensitivity of BChl-B850 needs further investigation.

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