Membrane Protein Stability: High Pressure Effects on the Structure and Chromophore-Binding Properties of the Light-Harvesting Complex LH2[†]

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ABSTRACT: Using the bacteriochlorophyll *a* (Bchl) cofactors as intrinsic probes to monitor changes in membrane protein structure, we investigate the response to high-pressure of the LH2 complexes from the photosynthetic bacteria *Rhodobacter sphaeroides* 2.4.1 and *Rhodopseudomonas acidophila* 10050. By FT-Raman spectroscopy, we demonstrate that high pressure does not induce significant distortion of the protein-bound 850 nm-absorbing bacteriochlorophyll molecules, or break of the hydrogen bond they are involved in. This indicates in particular that the oligomerization of the polypeptides is not perturbed up to 0.6 GPa. The pressure-induced changes in the Bchl absorption spectra are attributed to pigment−pigment interactions. In contrast, the loss of 800 nm-absorbing bacteriochlorophyll reflects pressure-induced alterations to the tertiary structure of the protein in proximity to the membrane/cytosol interface. This suggests that the LH2 protein does have two independent structural domains. The first domain is pressure independent and comprises mostly the C-terminal domain. The second domain located on the N-terminal side exhibits sensitivity to pressure and pH reminiscent of soluble proteins. The LH2 thus constitutes a suitable model system for studying in detail the stability of membrane-embedded hydrophobic helices and helices located at or close to the solvent/membrane interface.

Membrane proteins have been estimated to constitute at least 30−50% of the total number of proteins present in any organism. However, their physicochemical properties are still largely unknown, because of the many difficulties met upon, for example, overexpression and purification of this class of macromolecule and subsequent verification of functionality. The peripheral antenna of purple photosynthetic bacteria, or LH2,[1] 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. X-ray crystallography studies of the LH2 from *Rhodopseudomonas* (*Rps.) acidophila* 10050 have revealed the structural organization of this pigment−protein complex (*1*−*3*). This LH2 has a nonameric ring structure (Figure 1a). In these nonamers, each unit contains two small, single membrane-spanning, polypeptides, called α and *β*. The nine α-apoproteins form a hollow cylinder with the nine *β*-apoproteins arranged radially outside (*4*, *5*). To each α*β* pair, one carotenoid (rhodopin glucoside) and three bacteriochlorophyll *a* (Bchl) molecules are noncovalently attached. On the basis of weak crystallographic data (*3*), a putative second carotenoid molecule may be present; however, its presence, or lack of (*6*), needs to be investigated further. Most of the α-polypeptide/*β*-polypeptide interactions in LH2 occur close to the membrane/water interfaces, between both the C- and N-membrane-hugging termini (*3*), while the pigments (both Bchl and carotenoid molecules) mediate most of the α*β* 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 *β*-apoproteins (see Figure 1a) and form a ring of overlapping Bchl in close contact. These

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[1] Abbreviations: B800, B850, binding sites for bacteriochlorophyll *a* absorbing at 800 and 850 nm, respectively; B850 complex, Bchl-B800 depleted LH2 protein; Bchl, bacteriochlorophyll *a*; Bchl-B800 and -850, Bchl molecule in the B800 and B850 binding pockets, respectively; FT, Fourier transform; hwhm, half-width at half-maximum; LDAO, *N*,*N*-dimethyldodecylamine-*N*-oxide; LH, light-harvesting; Rb., Rhodobacter; RC, reaction center; Rps., Rhodopseudomonas.

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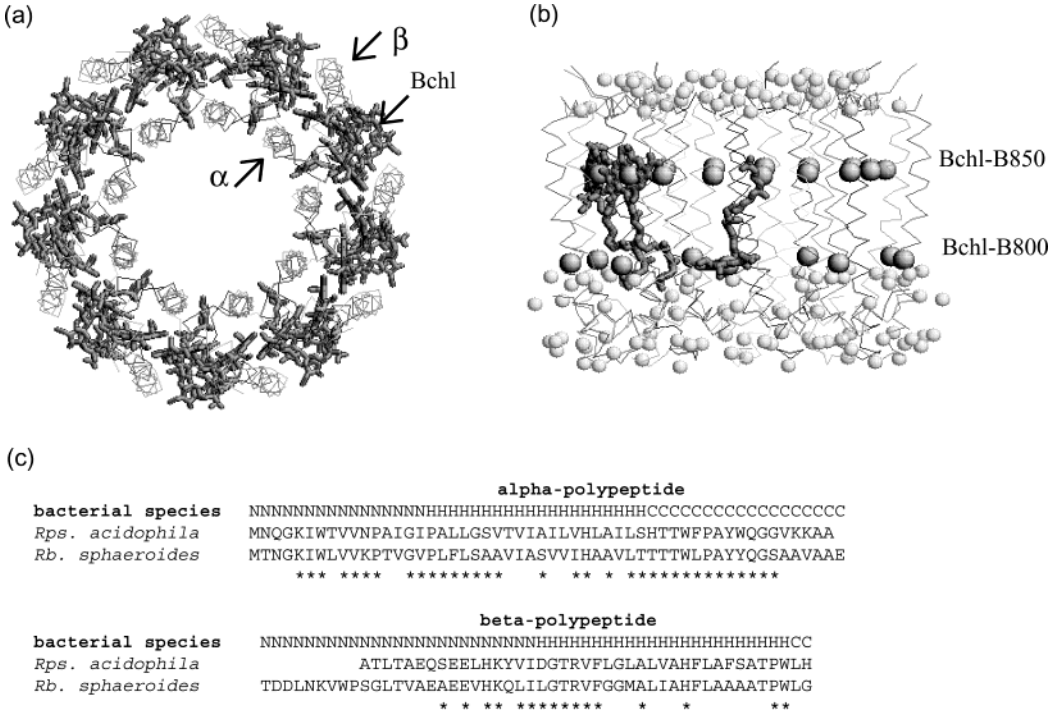

FIGURE 1: Schematic representation of the structure of the LH2 from *Rps. acidophila*, (a) parallel to the plane of the membrane with the location of the macrocycles of the bacteriochlorophylls shown, and (b) perpendicular to the plane of the membrane. The dark spheres represent the central Mg ions of the Bchl molecules and the light spheres represent the structured water molecules that are observed in the crystal structure. (c) Primary sequences of the α- and *β*-polypeptides from *Rps. acidophila* and *Rb. sphaeroides* aligned on highly conserved Bchl-binding His residue. Key: *, conserved residues in the LH2 family of proteins (see ref *45*); the C-, N- and hydrophobic membrane-spanning domains are marked by C, N, and H, respectively.

molecules absorb in the near-infrared at 850 nm and are known as the Bchl-B850 molecules. The other nine Bchl molecules lie toward the cytoplasmic side of the membrane (Figure 1b) and are located between the transmembrane helices of the *β*-apoproteins. These pigments exhibit spectroscopic properties close to monomeric Bchls (*7*). They absorb in the near-infrared at 800 nm, and are denoted 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 the Bchl-B800, it essentially depends on the interactions between the Bchl molecules with their specific microenvironment (*8*), while for the Bchl-B850 it results from a combination between Bchl/Bchl interactions and interactions between each Bchl and the surrounding protein (*9*−*11*). These interactions being very sensitive to localized structural (environmental) changes, the positions of the Q_y transitions thus constitute intrinsic molecular probes that are able to monitor the integrity of the LH2 structure. The electronic properties of LH2 yields information both on the structure of the Bchl binding sites within this protein (which in turns yields information on the tertiary structure of the polypeptides in the vicinity of these molecules) and indirectly (although highly persuasive) on its quaternary structure. As a result, this makes our LH2 family of proteins an ideal subject to study the stability of membrane-embedded hydrophobic α-helical membrane helices.

LH2 may be isolated from a variety of organisms. From the primary sequence of their polypeptides, it can be concluded that the LH2 proteins from *Rps. acidophila* and *Rhodobacter* (*Rb.*) *sphaeroides* have very similar secondary structure (Figure 1c). As in *Rps. acidophila*, the LH2 from *Rb. sphaeroides* is constituted from a ring of nine α/*β*-heterodimers (*12*). The carotenoid in this protein is spheroiden(on)e, depending on the exposure of the cell to oxygen during growth. These LH2 proteins have been the subject of most of the investigations into the molecular interactions within light-harvesting (LH) proteins as it lends itself readily to site-directed mutagenesis (*13*). There is no such genetic system available in *Rps. acidophila*, but methods for chemically modifying the LH2 proteins of this bacterium have been recently developed. For instance, the Bchl-B800 molecules in these LH2 can be selectively released, without perturbing the energy transfer between the carotenoids and Bchl-B850 pigments (*14*, *15*), nor causing significant structural changes to the B850 site (*16*).

Various antenna pigment−protein complexes from purple photosynthetic bacteria have been previously studied at high pressure, both at ambient temperature (*17*−*20*) and at cryogenic temperatures (*21*, *22*). These studies have shown that increasing the pressure causes a substantial red shift and broadening of the long wavelength Q_y absorption band both in LH1 and in LH2. In this work, we have combined both FT-Raman and absorption spectroscopies, measured at room temperature, to study the LH2 from *Rb. sphaeroides* at high hydrostatic pressures. We have also compared the pressure sensitivity, as probed by electronic absorption spectroscopy, of the LH2 proteins from *Rps. acidophila* and *Rb. sphaeroides* in their native conformations and after selective removal of the Bchl-B800 molecules.

EXPERIMENTAL PROCEDURES

Membrane Preparation and Protein Isolation. Rps. acidophila 10050 and *Rb. sphaeroides* 2.4.1 were grown

anaerobically at 30 °C in Pfenning's (*23*) and Böse's media (*24*), respectively. *Rb. sphaeroides* 2.4.1 cells were cultured under strict anaerobic conditions or grown semi-aerobically, which ensured that the carotenoid present in LH2 is spheroidene (97% spheroidene, 3% spheroidenone) and spheroidenone, respectively. Cells were harvested by centrifugation. Rupturing the whole cells in a French press then allowed the isolation of the photosynthetic membranes after centrifugation. The membranes were solubilized using *N*,*N*-dimethyldodecylamine-*N*-oxide (LDAO), the LH2 proteins purified as previously described (*25*). When required, the Bchl-B800 molecules were removed as previously described (*14*, *26*). For use in the high-pressure optical cell, LH2 samples were prepared in a buffer containing 0.035% LDAO, 50 mM NaCl, 20 mM Tris·Cl, pH 8.0.

High-Pressure Optical Cell. The high-pressure optical cell used in this work reproducibly generates pressures up to a maximum in excess of 1 GPa and has been described previously (*18*, *20*). In summary, it consisted of a piston-cylinder type high-pressure optical cell with three sapphire windows. The protein sample under study was inserted into a separate sample cell. The sample cell has two windows, 2−3 mm apart, with one window fixed and another movable like a piston to adapt to the pressure variations. Pressure was generated inside the cell by a small hydraulic press, and was transmitted to the sample by a liquid (glycerol−water) phase. A manganin-wire gauge was used to measure pressure in the cell and calibrate the equipment. Samples were allowed to equilibrate for 30 min after an increase in the pressure. Reversibility of the pressure-induced effects on LH2 proteins was checked systematically by measuring absorption and/or FT-Raman spectra upon pressure release, after the pressure maximum had been applied.

Spectroscopy. Room temperature absorption spectra, at atmospheric pressure and under pressure, were recorded using a Cary 5 spectrophotometer (Varian plc, Sidney). The pressure cycle used was 0.0001 (atmospheric pressure), 0.03, 0.2, 0.4, 0.6, 0.6 (after a further delay of 90 min), 0.3, and then 0.03 GPa. These spectra were corrected by subtracting, at each applied pressure, a reference spectrum corresponding to that of the buffer alone in the pressure cell.

FT-Raman spectra were recorded using a Bruker IFS 66 interferometer coupled to a Bruker FRA 106 Raman module equipped with a continuous Nd:YAG laser, as described previously (*19*). For pressure measurements, all spectra were recorded at room temperature with backscattering geometry. Depending on sample conditions, spectra were the result of 3000 to 10000 co-added interferograms. This corresponds to between 100 and 225 min of data acquisition. The main sapphire vibrational mode, observed at 417 cm^{-1}, displays a pressure sensitivity of 1.4 cm^{-1} GPa^{-1} for the pressure range used in this work (*19*), and was used as an internal pressure standard.

RESULTS

Increasing pressure to 0.6 GPa induces a large redshift and a clear broadening of the Q_y electronic transition of the Bchl-850 molecules of the spheroidenone-containing LH2 protein from *Rb. sphaeroides* (Figure 2a). The Q_y-Bchl-850 transition has a quasi-linear relationship with pressure, with the pressure dependence of the absorption maximum (hence-

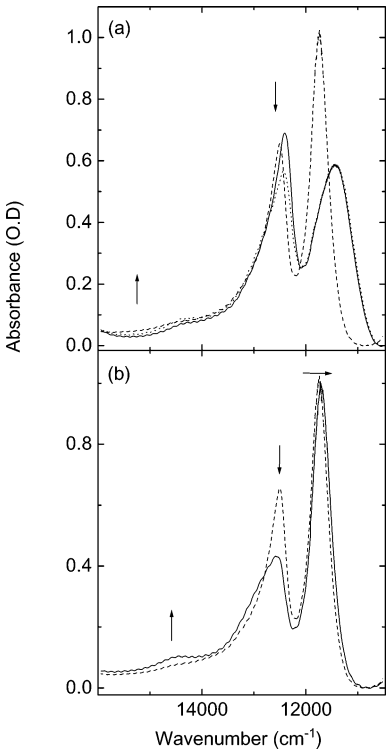

FIGURE 2: Selective temporal loss of the Bchl-B800 molecules from the LH2 complex from *Rb. sphaeroides* containing spheroidenone. (a) Absorption spectra at the minimal applied pressure of 0.03 GPa (dashed line), after 15 min at 0.6 GPa (solid line) and 90 min (dotted line). (b) Comparison between the initial (broken line) and final (solid line) absorption spectra at the minimal applied pressure (0.03 GPa). In both plots, the arrows highlight the major evolution of absorption intensities and positions.

forth referred to as the slope) of −639 cm^{-1} GPa^{-1}. It has been proposed that the pressure-induced shift of proteins may be composed of both linear and quadratic terms. We shall ignore the latter term here as its influence is small (*17*, *20*). The Bchl-B800 transition exhibits a much-reduced slope having a quasi-linear relationship of only some -203 ± 50 cm^{-1} GPa^{-1}. Shown in Figure 2a are absorption spectra taken 15 min (solid line) and 90 min (dotted line) after an increase in pressure to 0.6 GPa. The red-shifted 850 nm transition is unaltered in both shape and intensity after 90 min. Although the extinction coefficient decreases under pressure, the integrated area stays the same, as a difference spectrum between 0.6 GPa^{-1} and minimal pressure indicates a discrepancy of only 3.6%. Consequently, the apparent loss of B850 intensity is indicative of changes in the specific structures of the Q_y transitions and not an actual loss of Bchl-B850 molecules under pressure. However, there is a significant decrease in the intensity of the 800 nm transition, associated with an increase in absorption between 680 and 770 nm (Figure 2a, arrow). This absorption range is expected to contribute the Q_y transition of Bchl released from the protein (at ca. 770 nm) and of oxidized Bchl in solution (at ca. 680 nm) (*10*). Thus, we conclude that the change in the LH2 absorption spectrum is due to the perturbation of the protein-binding site of the Bchl-B800 molecules, which leads to the release of these molecules in the aqueous solvent and their subsequent oxidation. This is further emphasized by the absorption differences between the initial (dashed line) and final (solid line) measurements under minimal applied hydrostatic pressure (Figure 2b). After the pressure cycle,

Table 1: Evolution of Selected Fingerprint Raman Vibrational Modes as a Function of Applied Pressure[a]

mode/pressure (GPa)	0.0001 (atm)	0.2	0.3	0.4	0.6	slope ($\pm$ cm^{-1} GPa^{-1})	slope error
R_6							
R_5							
R_4							
R_3	1442.9	1442.9	1442.9	1443.3	1443.7	3.5	1.5
R_2							
R_1	1604.7	1604.9	1604.9	1604.9	1607.0	1.4	0.5
Bchl-B800 ν2C=O	1628*	*	*	*	*		
Bchl-B850 ν2C=O	1627*	*	*	*	*		
Bchl-B850 ν2C=O	1634.6	1633.7	1633.6	1633.6	1632.9	2.6	0.4
Bchl-B850 ν9C=O	1651.2	1651.2	1651.2	1650.6	1648.9	3.8	2.2
Bchl-B850 ν9C=O	1679.3	1678.2	1679.0	1676.6	1677.2	4.1	1.9
Bchl-B800 ν9C=O	1699.5	1698.4	1697.7	1701.2	1701.2	3.8	3.5

[a] The modes marked with an asterisk (*) are a result of converging Raman lines (see text). The slope values in parentheses are best-guess estimates due to convolution of some of the Raman modes. The samples contain a number of intense carotenoid Raman bands that mask certain Bchl R-conformation Raman lines. $\lambda_{ext} = 1064$ nm, $T = 293$ K.

nearly half of the intensity of the Q_y transition of the Bchl-B800 is lost, while the transition at 850 nm has conserved its intensity and half width at half maximum (hwhm). The latter is very slightly red shifted, similarly to what is observed upon selective removal of the Bchl-B800 from LH2 proteins (*26*). This response of the Bchl-850 molecules is thus very likely associated with the loss of Bchl-B800 pigment loss during the pressure cycle. It may be used to evaluate whether the loss of the Bchl-B800 occurs homogeneously among the rings, or whether some rings lose all the Bchl-B800 while others stay intact: in the latter case, a broadening of the Q_y transition of the Bchl-B850 should be observed at low temperature. It is not (data not shown), and we thus conclude that the loss of BChl-B800 is homogeneous among the LH2 rings.

FT-Raman spectra of the spheroidenone-containing LH2 protein from *Rb. sphaeroides* were recorded at various applied pressures. This vibrational technique provides information both on the conformation of, and on the intermolecular interactions assumed by, the Bchl bound to photosynthetic complex (*27*). The majority of the Raman modes observed undergo only slight, or negligible, pressure-induced displacements of the order of up to 5 cm^{-1} GPa^{-1} (not shown). Distortions of the Bchl macrocycle may be very accurately evaluated by this technique, from the changes in frequency of six modes, denoted R_1 to R_6 (*28*). Due to the presence of carotenoid contributions in the spectra, the majority of the bands arising from these modes are masked; however, those arising from R_1 (νCaCm(α,β,γ,δ)) and R_3 (νCaN,νCaCm) are clearly visible in the FT-Raman spectra. These bands do not experience larger shifts than the other FT-Raman bands, indicating a minimal variance in Bchl conformation as a function of applied pressure (Table 1). In Figure 3 are shown the 1550 to 1750 cm^{-1} regions of the FT-Raman spectra obtained at different pressures, normalized on the Bchl-B850 contributions. The bands contributing between 1620 and 1710 cm^{-1} arise from the carbonyl stretching modes of the acetyl and keto carbonyl groups of the Bchl molecules. The Bchl-B850 molecules contribute at about 1627, 1632, 1651, and 1678 cm^{-1} (*9*, *29*), while the contributions of Bchl-B800 are located at about 1626 and 1699 cm^{-1} (*9*, *16*, *30*, *31*). Upon an increase of hydrostatic pressure, the 1699 cm^{-1} band (which arises from the stretching mode of the keto carbonyl group of the Bchl-B800 molecule) steadily becomes weaker, its position remaining

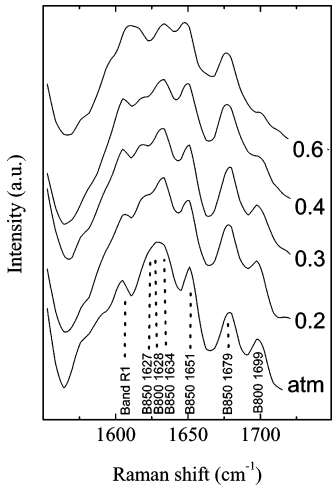

FIGURE 3: The effect of pressure on the high-frequency region (1575−1750 cm^{-1}) of the room temperature FT Raman spectrum of bacteriochlorophyll *a* molecules in the LH2 protein, in preresonance with the Q_y electronic transition of the Bchl-B850 molecules. The sequence of the FT-Raman data collection was 0.0001, 0.2, 0.4, 0.6 and 0.3 GPa; $\lambda_{ext} = 1064$ nm.

invariant. This is easily explained by pressure-induced changes in resonance conditions. As pressure increases the position of the Q_y of the Bchl-B850 shifts toward the red, therefore, inducing an increase of the preresonance Raman signal of these molecules (which is observed using the 417 cm^{-1} sapphire band as an intensity standard). Meanwhile, the Q_y transition of the Bchl-B800 experiences only a slight red shift. Thus, the preresonance Raman signal of the latter molecule remains nearly constant and its relative contribution to the total spectrum decreases as pressure is increased. In addition, the progressive bleaching of the transition at 800 nm is expected to result in a progressive loss of the contributions of the Bchl-B800 from the FT-Raman spectra of LH2. Because of the combination of these effects, at 0.6 GPa, the majority of the FT-Raman contributions indeed arise from mostly the Bchl-B850 molecules. FT-Raman spectra recorded at this pressure are not dramatically different from those recorded at atmospheric pressure, other than the reduction of the contributions of the Bchl-B800 molecules. In particular, the 1634 cm^{-1} band, which arises from the stretching mode of the acetyl carbonyl, in interaction with Tyr alpha 44, is not affected by pressure increase. An exception to this concerns the band contributing at 1627 cm^{-1}, arising from the acetyl carbonyl groups of the Bchl

Table 2: Comparison of Pressure-Sensitivities[a] for the Different LH2 Proteins Investigated in This Work

protein	Bchl-800 peak	Bchl-800 hwhm	Bchl-850 peak	Bchl-850 hwhm
Rps. acidophila LH2, pH 8.0	-194 ± 21	35 ± 26	-763 ± 54	213 ± 7
Rb. sphaeroides LH2, pH 8.0	-197 ± 58	53 ± 84	-669 ± 63	294 ± 18
Rps. acidophila LH2, pH 4.75	-153 ± 74	139 ± 58	-846 ± 64	258 ± 7
Rps. acidophila B850 complex, pH 8.0			-1030 ± 19	259 ± 44
Rb. sphaeroides B850 complex, pH 8.0			-995 ± 5	275 ± 289

[a] Measured in cm^{-1} GPa^{-1} Units.

bound to the beta polypeptide in interaction with Trp alpha 45 (*4*, *30*). This band shifts continuously to ca. 1620 cm^{-1} between atmospheric pressure and 0.6 GPa, partially merging with the conformation band R_1 (*28*). Such a downshift could arise from slight strengthening of the hydrogen bond this acetyl carbonyl group is involved in, or possibly from changes in the electrostatic properties of the Bchl molecule environment. In any case, these changes are small, of the same order of magnitude as the shifts observed in FT-Raman spectra of the bacterial reaction centers upon lowering the temperature to 10 K (*32*). The FT-Raman spectra recorded at 0.6 GPa thus indicate unambiguously that the structures of the nine Bchl-B850 binding sites are nearly insensitive to the applied hydrostatic pressure.

We examined the pressure sensitivity of the electronic absorption properties of the LH2 proteins fromspheroidene-containing *Rb. sphaeroides* 2.4.1 and from *Rps. acidophila* 10050. These proteins exhibit clearly different pressure-induced absorption changes, especially in the B800 range (Figure 4). The energy-shift and broadening experienced by the transition of the Bchl-B850 molecules in these complexes upon pressure application are of the same order of magnitude, but not identical (Table 2). The initial slopes for the Bchl-B800 molecules in both complexes are nearly identical. It is of note that a change in carotenoid end-grouping has no measurable effect on the pressure sensitivity of LH2 from *Rb. sphaeroides*. During a pressure cycle, there is only a very limited loss (~10%) of Bchl-B800 molecules in the LH2 proteins from *Rps. acidophila* (Figure 4a). The same pressure cycle has a much greater effect on LH2 from *Rb. sphaeroides* (Figures 2b and 4b), where about 40% of the Bchl-B800 molecules are dislodged from their binding site. When submitting LH2 from *Rps. acidophila* to the same pressure cycle at pH 4.75, a large reduction of the electronic transition of the Bchl-B800 is observed, indicating that most of these molecules have left their protein-binding site (Figure 4c). This is accompanied by a small decrease in intensity of the transition of the Bchl-B850 molecules, indicating that most of them have not been perturbed during the pressure cycle.

The Bchl-B800 molecules from each protein may be selectively removed by established biochemical techniques (*14*, *26*). Figure 5 shows the pressure response of the B850 complex from *Rps. acidophila* when probed by absorption spectroscopy. The transition at 850 nm of these complexes exhibits a higher pressure sensitivity than that of the untreated samples (Table 2). The LH2 from *Rps. acidophila* under acidic conditions (Figure 5, inset) exhibits an intermediate pressure sensitivity, somewhere between the native LH2 and B850 complex, presumably resulting from the progressive loss of most of the Bchl-B800 molecules during the pressure cycle.

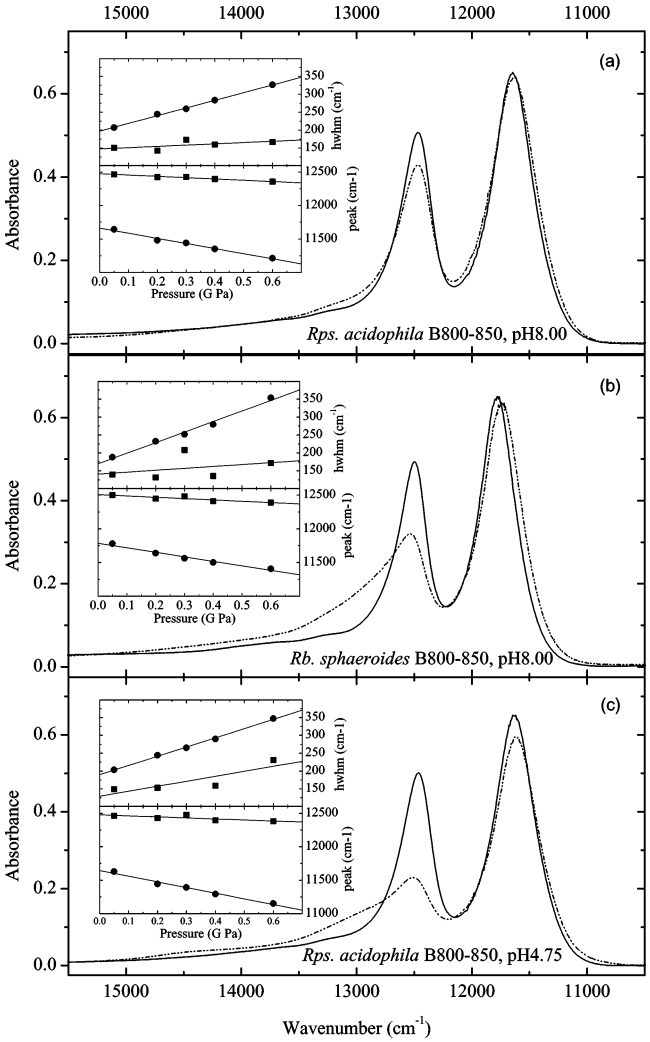

FIGURE 4: Comparison of the pressure-induced spectral changes observed in different LH2 proteins. (a) *Rps. acidophila* at pH 8.0, (b) spheroidene-containing *Rb. sphaeroides*, pH 8.0, and (c) *Rps. acidophila*, pH 4.75. In each case, the plotted spectra at 0.03 GPa are taken at the beginning (solid line) and end (broken line) of the cycle of high pressure (in the order of 0.03, 0.1, 0.2, 0.4, 0.6, 0.6 (delay of 90 min), 0.3, and 0.03 GPa). The insets represent the trend in peak position and hwhm for each electronic transition. The insets illustrate the location of peak position and the half width at half maximum (hwhm) (calculated on the lower energy side) for each absorption band.

DISCUSSION

Effect of Pressure on the Quaternary Structure of LH2s. In this work, we have studied the response of native LH2 proteins from *Rps. acidophila* and *Rb. sphaeroides* to high pressures. Absorption experiments suggest that no dissociation of the nonameric, annular structure of LH2 occurs between atmospheric pressure and 0.6 GPa. Dissociation of

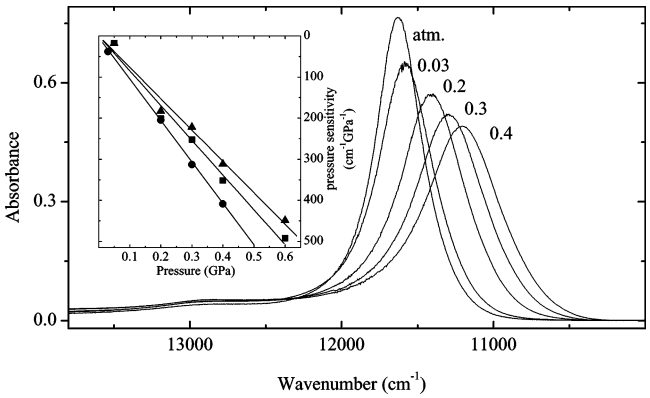

FIGURE 5: Pressure-induced structural changes observed in the B850 complex from *Rps. acidophila*. The numbers beside the plotted spectra are the pressure values in GPa; atm is atmospheric pressure. The inset compares the pressure sensitivity of the Q_y-BchlB850 transition of the B850 complex (solid circles) with that of the LH2 at pH 4.75 (solid squares) and pH 8.00 (solid triangles).

the rings should result in a net loss of at least part of the interactions between the different Bchl-B850 molecules, which should in turn induce a blue shift of their infrared transition. By contrast, a continuous red shift of this transition is observed upon pressure increase. FT-Raman experiments provide more direct evidence that the annular structure of the LH2 is maintained at high pressure. They show that, during the pressure cycle, the interactions between the Bchl-B850 and the neighboring amino acid side chains at position α_{44} and α_{45} remain nearly unchanged. These interactions make a link between neighboring $\alpha\beta$ subunit in the ring, and the dissociation of the annular structure should result in their breakage. That an oligomeric protein does not dissociate at pressures as high at 0.6 GPa is quite an unusual result, as most of the multichain soluble proteins dissociate below 0.2 GPa (for a review see ref *33*). Experiments performed with RC-LH1 protein complexes have led to a similar suggestion (*19*). However, the latter were performed on RC-LH1 complexes in membranes, whereas these present experiments have been performed with LH2 proteins in detergent micelles. Moreover, the structure of the LH2 protein is very likely to be less compact than that of RC-LH1, with a relatively much larger surface interacting with the surrounding lipids and/or detergent molecules (*34*−*36*). The absence of ring dissociation at high hydrostatic pressure indicates that the associated LH2 ring represents a structure of minimal volume as compared to the dissociated polypeptides solvated by either the detergent or the water molecules. Dissociation of oligomeric proteins has been attributed to negative volume changes due to two major phenomena, electrostriction and formation of clathrates around hydrophobic residues. Due to the highly apolar nature of the LH2 polypeptides, and the absence of ion pairs in the structure, electrostriction should not contribute to the volume changes during LH2 dissociation. Formation of clathrates around hydrophobic residues is expected to result in a negative volume change through stronger and shorter van der Waals interactions resulting from protein−water interaction (*37*). In the case of glycophorin *a* dimer, it was shown that the packing of the hydrophobic side chains in the interaction surface between the membrane helices was nearly optimal. It is thus possible that no negative volume change can be achieved by optimizing van der Waals interactions through the dissociation the LH2 subunits, neither with the surrounding detergent, nor with water molecules. It is of note that the question is still open about the origin of the negative volume changes during soluble protein unfolding by hydrostatic pressure. In particular, whether solvating hydrophobic amino acids should result in a *positive* or *negative* volume change is still a matter of debate (see ref *38* for a review). Our present experiments, as well as those from refs *19* and *39*, strongly suggest that exposure of apolar amino acid should not result in a large negative volume change, as no unfolding of the highly hydrophobic light-harvesting or reaction center proteins is observed at high hydrostatic pressure.

Effects of Pressure on the Electronic Properties of LH2. From the FT-Raman experiment, it may be concluded that neither the conformation of the Bchl-B850, nor their interactions with the surrounding protein are modified at high pressure. The strengthening of the hydrogen bond between the bound Bchl and Trp alpha 45 cannot, by itself, explain the changes in the electronic absorption of the complex (*40*). The changes in the position (and width) of their lower energy absorption must thus be mostly attributed to changes in the interactions between the Bchl-B850 molecules, arising from very small changes in distance between the molecules and/or changes in the extent of the overlap of their molecular orbitals. It is of note that the extent of the variations of both the position and the width of these transitions are different from protein to protein. The fact that these variations are larger in proteins devoid of Bchl-B800 may suggest that they depend on the compressibility of the protein structure, the latter being expected to be higher after the Bchl-B800 molecules have been removed. However, Stark measurements have previously shown that the electronic structure (and, in particular, the charge-transfer character) of the 850 nm Q_y-Bchl transition of LH2 varies between species (e.g., *Rps. acidophila*, *Rb. sphaeroides*, and *Rhodospirillum molischianum*) (*41*). The different sensitivities to pressure of the transitions at 850 nm of the different complexes likely depend on their precise electronic structures, rather than being directly connected to the protein compressibility. This conclusion is also supported by the fact that there is no obvious relationship between the extent of the red shift and the change in width experienced by this transition upon pressure application (Table 2).

Existence of Domains of Stability in the LH2 Structure. Although the structure of the LH2 protein is not affected by pressure at the level of the Bchl-B850 molecules, it is clear that in the case of LH2 from *Rb. sphaeroides*, and of LH2 of *Rps. acidophila* at pH 4.75, increasing pressure results in the loss of sizable amounts of Bchl-B800. Such a Bchl release indicates a partial opening of the binding sites of these molecules to solvent when pressure is applied. The binding site of these molecules is provided by the amino-terminal part of the alpha polypeptide, which enters the membrane between residues 1 and 6, so that the carboxy modified $_{\alpha}Met_1$ amino-terminal group of this polypeptide coordinates their central magnesium atoms (*3*). The first amino acids of the alpha polypeptide are mostly hydrophilic (sequence Asn-Gln-Gly-Lys-Ile-Trp-Thr), while the following ones, supposed to be membrane-embedded, are quite hydrophobic (sequence Val-Val-Pro-Ala-Ile-Gly-Ile). It is of note that Bchl is a quite large molecule (the diameter of the macrocycle is about 10 Å). The release of the Bchl-B800

likely may occur as a result of a large motion (or a large increase in flexibility) in the terminal fragment, which is, in the native protein, interacting with the surrounding β polypeptides and the Bchl-B800. Such reorganization, upon the application of pressure, must involve a negative volume change. The pressure at which this phenomenon is observed is, in the case of LH2 from *Rps. acidophila*, pH dependent. It is thus reminiscent of what is observed with soluble proteins, but it has an extremely limited impact on the structure of binding site of the Bchl-B850. Recently, the detergent envelope that surrounds the LH2 complex was characterized (*36*). It covers only 30−35 Å of the protein, which corresponds to the position of the most hydrophobic core, a situation that is quite typical of membrane proteins (*42*−*44*). This places the Bchl-B800 molecules (which are nearly parallel to the in vivo lipid surface) in an environment that is more in proximity to the solvent phase (as indirectly evidenced by the presence of crystallographic water molecules that are near to these bacteriochlorophyll molecules). In light of the proposed depth of the detergent sheath, the amino-terminal end of the alpha polypeptide will be positioned between 6 and 8 Å from the in vitro detergent/solvent (H_2O) interface and therefore close to the hydrophobic/hydrophilic interface, which may explain why this particular membrane polypeptide segment may indeed exhibit properties very similar to those observed for soluble proteins. We thus conclude that the structure of the LH2 is constituted of at least two independent domains, exhibiting a different response to application of high hydrostatic pressures. For the same detergent/buffer/pH environment the Bchl-B800 binding site in *Rps. acidophila* shows greater resistance to pressure than *Rb. sphaeroides* (Figure 4 and Table 2), indicating that the stability of the amino-terminal structure of the protein is dependent on the primary structure. There are very few significant changes between the sequences of the alpha polypeptides of these two LH2 (only at position 11, Lys for *Rb. sphaeroides* and Asn for *Rps. acidophila*). The differences in the beta polypeptides sequence are larger, but their analysis does not lead to any clear hypothesis accounting for the changes in response to pressure of these two proteins.

Clearly, high-pressure measurements can provide new insights for understanding the various elements that help to stabilize LH2 (and LH1) proteins. The next step may be achieved by comparing in detail the response to high pressures of LH2 either isolated, or still embedded in membranes, from genetically engineered bacteria in which only this type of LH protein is synthesized. The study of the stability of the LH2 within their native membrane and isolated in detergent will be important for understanding to which extent the small amphiphile molecules can preserve the structure and stability of related membrane proteins during protein solubilization, purification, and subsequent crystallographic trials. Of obvious interest will also be the study of genetically engineered LH2, in which alteration of the amino acid sequence will introduce defects of compacticity, to understand in detail the response of these proteins to high hydrostatic pressures.

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REFERENCES

1. Cogdell, R. J., Isaacs, N. W., Freer, A. A., Arrelano, J., Howard, T. D., Papiz, M. Z., Hawthornthwaite-Lawless, A. M., and Prince, S. (1997) *Prog. Biophys. Mol. Biol. 68*, 1−27.
2. Cogdell, R. J., Isaacs, N. W., Freer, A. A., Arrelano, J., Howard, T. D., Papiz, M. Z., Hawthornthwaite-Lawless, A. M., and Prince, S. (1998) *Prog. Biophys. Mol. Biol. 70*, I−V.
3. Papiz, M. Z., Prince, S. M., Howard, T., Cogdell, R. J., and Isaacs, N. W. (2003) *J. Mol. Biol. 326*, 1523−1538.
4. McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., and Isaacs, N. W. (1995) *Nature 374*, 517−521.
5. Freer, A., Prince, S., Sauer, K., Papiz, M., Hawthornthwaite-Lawless, A., McDermott, G., Cogdell, R., and Isaacs, N. W. (1996) *Structure (London) 4*, 449−462.
6. Arellano, J. B., Raju, B. B., Naqvi, K. R., and Gillbro, T. (1998) *Photochem. Photobiol. 68*, 84−87.
7. Van Grondelle, R. (1985) *Biochim. Biophys. Acta 811*, 147−195.
8. Gall, A., Fowler, G. J. S., Hunter, C. N., and Robert, B. (1997) *Biochemistry 36*, 16282−16287.
9. Fowler, G. J. S., Sockalingum, G. D., Robert, B., and Hunter, C. N. (1994) *Biochem. J. 299*, 695−700.
10. Sturgis, J. N., and Robert, B. (1994) *J. Mol. Biol. 238*, 445−454.
11. Sturgis, J. N., Olsen, J. D., Robert, B., and Hunter, C. N. (1997) *Biochemistry 36*, 2772−2778.
12. Walz, T., Jamieson, S. J., Bowers, C. M., Bullough, P. A., and Hunter, C. N. (1998) *J. Mol. Biol. 282*, 833−845.
13. Hunter, C. N. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blakenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 473−501, Kluwer Academic Publishers, Dordrecht.
14. Fraser, N., J., Dominy, P. J., Ücker, B., Simonin, I., Scheer, H., and Cogdell, R. J. (1999) *Biochemistry 38*, 9684−9692.
15. Fraser, N. J. (1998) Ph.D. Thesis, University of Glasgow, Glasgow.
16. Gall, A., Fraser, N. J., Bellissent-Funel, M.-C., Scheer, H., Robert, B., and Cogdell, R. J. (1999) *FEBS Lett. 449*, 269−272.
17. Freiberg, A., Ellervee, A., Kukk, P., Laisaar, A., Tars, M., and Timpmann, K. (1993) *Chem. Phys. Lett. 214*, 10−16.
18. Tars, M., Ellervee, A., Kukk, P., Laisaar, A., Saarnak, A., and Freiberg, A. (1994) *Liet. Fiz. Z. 34*, 320−328.
19. Sturgis, J. N., Gall, A., Ellervee, A., Freiberg, A., and Robert, B. (1998) *Biochemistry 37*, 14875−14880.
20. Timpmann, K., Ellervee, A., Pullerits, T., Ruus, R., Sundstrom, V., and Freiberg, A. (2001) *J. Phys. Chem. B 105*, 8436−8444.
21. Reddy, N. R. S., Wu, H.-M., Jankowiak, R., Picorel, R., Cogdell, R. J., and Small, G. J. (1996) *Photosynth. Res. 48*, 277−289.
22. Wu, H.-M., Ratsep, M., Jankowiak, R., Cogdell, R. J., and Small, G. J. (1997) *J. Phys. Chem. B 101*, 7641−7653.
23. Pfenning, N. (1969) *J. Bacteriol. 99*, 597−602.
24. Böse, S. K. (1963) in *Bacterial Photosynthesis* (Gest, H., Pietro, A., and Vermon, L. P., Eds.) pp 501−510, The Antioch Press, Yellow Springs, Ohio.
25. Gall, A., and Robert, B. (1999) *Biochemistry 38*, 5185−5190.
26. Bandilla, M., Ucker, B., Ram, M., Simonin, I., Gelhaye, E., McDermott, G., Cogdell, R. J., and Scheer, H. (1998) *Biochim. Biophys. Acta 1364*, 390−402.
27. Robert, B. (1996) in *Biophysical Techniques in Photosynthesis* (Amez, A., and Hoff, A., Eds.) pp 161−276, Kluwer Academic Publisher, Amsterdam.
28. Näveke, A., Lapouge, K., Sturgis, J. N., Hartwich, G., Simonin, I., Scheer, H., and Robert, B. (1997) *J. Raman Spectrosc. 28*, 599−604.
29. Sturgis, J. N., Jirsakova, V., Reiss-Husson, F., Cogdell, R. J., and Robert, B. (1995) *Biochemistry 34*, 517−523.
30. Robert, B., and Lutz, M. (1985) *Biochim. Biophys. Acta 807*, 10−23.
31. Gall, A., Robert, B., Cogdell, R. J., Bellissent-Funel, M. C., and Fraser, N. J. (2001) *FEBS Lett 491*, 143−147.
32. Ivancich, A., Lutz, M., and Mattioli, T. A. (1997) *Biochemistry 36*, 3242−3253.
33. Boonyaratanakornkit, B. B., Park, C. B., and Clark, D. S. (2002) *Biochim. Biophys. Acta 1595*, 235−249.
34. Damjanovic, A., Kosztin, I., Kleinekathofer, U., and Schulten, K. (2002) *Phys. Rev. E 65*, 031919/1−031919/24.
35. Hu, X. C., Ritz, T., Damjanovic, A., Autenrieth, F., and Schulten, K. (2002) *Q. Rev. Biophys. 35*, 1−62.

36. Prince, S. M., Howard, T. D., Myles, D. A. A., Wilkinson, C., Papiz, M. Z., Freer, A. A., Cogdell, R. J., and Isaacs, N. W. (2003) *J. Mol. Biol. 326*, 307−315.
37. Silva, J. L., and Weber, G. (1993) *Annu. Rev. Phys. Chem. 44*, 89−113.
38. Royer, C. A. (2002) *Biochim. Biophys. Acta 1595*, 201−209.
39. Gall, A., Ellervee, A., Bellissent-Funel, M. C., Robert, B., and Freiberg, A. (2001) *Biophys. J. 80*, 1487−1497.
40. Sturgis, J. N., and Robert, B. (1997) *J. Phys. Chem. B 101*, 7227−7231.
41. Beekman, L. M. P., Frese, R. N., Fowler, G. J. S., Ortiz de Zarate, I., Cogdell, R. J., van Stokkum, I., Hunter, C. N., and van Grondelle, R. (1997) *J. Phys. Chem. B 101*, 7293−7301.
42. Roth, M., Lewit-Bentley, A., Michel, H., Deisenhofer, J., Huber, R., and Oesterhelt, D. (1989) *Nature 340*, 659−626.
43. Roth, M., Arnoux, B., Ducruix, A., and Reiss-Husson, F. (1991) *Biochemistry 30*, 9403−9413.
44. Penel, S., Pebay-Peyroula, E., Rosenbusch, J., Rummel, G., Schirmer, T., and Timmins, P. A. (1998) *Biochimie 80*, 543−551.
45. Zuber, H., and Cogdell, R. J. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blakenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 315−348, Kluwer Academic Publishers, Dordrecht.

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