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High-pressure modulation of the structure of the bacterial photochemical reaction center at physiological and cryogenic temperatures

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Abstract
The optical absorption and fluorescence response to external high pressure of the reaction center membrane chromoprotein complex from the wild-type non-sulfur photosynthetic bacterium Rhodobacter sphaeroides was investigated using the native pigment cofactors as local molecular probes of the reaction center structure at physiological (ambient) and cryogenic (79 K) temperatures. In detergent-purified complexes at ambient temperature, abrupt blue shift and accompanied broadening of the special pair band was observed at about 265 MPa. These reversible in pressure features were assigned to a pressure-induced rupture of a lone hydrogen bond that binds the photo-chemically active L-branch primary electron donor bacteriochlorophyll cofactor to the surrounding protein scaffold. In native membrane-protected complexes the hydrogen bond rupture appeared significantly restricted and occurred close to about 500 MPa. The free energy change associated with the rupture of the special pair hydrogen bond in isolate complexes was estimated to be equal to about 12 kJ mol⁻¹. In frozen samples at cryogenic temperatures the hydrogen bond remained apparently intact up to the maximum utilized pressure of 600 MPa. In this case, however, heterogeneous spectral response of the cofactors from the L-and M-branches was observed due to anisotropic build-up of the protein structure. While in solid phase, the special pair fluorescence as a function of pressure exactly followed the respective absorption spectrum at a constant Stokes shift, at ambient temperature, the two paths began to deviate strongly from one other at the hydrogen bond rupture pressure. This effect was tentatively interpreted by different emission properties of hydrogen-bound and hydrogen-unbound special pair exciton states.

Keywords: high pressure, photosynthesis, membrane protein stability, hydrogen bond, reaction center, Rhodobacter sphaeroides, cryogenic temperature

(Some figures may appear in colour only in the online journal)

1. Introduction
We have previously demonstrated that high pressure optical barospectroscopy combined with genetic engineering of a chromoprotein is a promising approach for studying poorly understood aspects of the roles of hydrogen (H-) bonds in membrane proteins [2–5]. An important element of this advance was suitable selection of the sample proteins such as light-harvesting (LH) complexes of photosynthetic purple bacteria with innate bacteriochlorophyll-a (BChl) pigment chromophores that may be used as local optical probes of the
H-bond interactions occurring in the chromophore binding pockets of the proteins. A comparison of the absorption spectra for the cyclic core LH complex (LH1) and two peripheral LH complexes (LH2 and LH3) in native membranes and in detergent environments revealed that the packing of the pigments in membrane environments is one of the significant stabilizing factors for these proteins. Whilst the membrane-bound LH complexes demonstrated high resilience to pressures as high as \( \geq 1 \text{ GPa} \), discontinuous band shifts were observed at much lower pressures for detergent-solubilized complexes. The irregular band shifts were accompanied by band broadening. These pressure effects, assigned to a disruption of H-bonds, were then analyzed for the estimation of H-bond energies in the membrane protein complexes [2, 4].

The early measurements described obviously probed a simultaneous rupture of multiple H-bonds in the cyclic structures of LH1 and LH2 complexes. To address the important question of whether the multiple H-bonds contribute to protein stability cooperatively or additively, we recently studied barospectroscopically the wild-type (WT) and mutant reaction center (RC) protein complexes from *Rhodobacter (Rba.) sphaeroides*, which possessed various number of H-bonds to the primary donor of electrons (see below) of the RC. The results of this study appeared to confirm cooperative mechanism of the H-bond rupture in LH complexes.

As reviewed in [6], most high-pressure studies of bacterial RCs have been carried on at ambient temperatures [7–15], and only a few at cryogenic temperatures. This disproportion is understandable because the proteins at ambient temperature are in flexible, close to native solvent surroundings, whilst at low temperatures they are confined in rigid solid environment. Nevertheless, since pressure and temperature are two major thermodynamic parameters, it is of basic interest to compare the response of the RC structure on high pressure at contrasting physiological and cryogenic temperatures. In the present work we investigated the dependence of absorption as well as fluorescence spectra of WT RCs on high external pressure reaching 600 MPa. This pressure was applied hydrostatically to the liquid samples kept at ambient temperature of 295 K \( \pm 1 \text{ K} \) as well as to the solid samples at cryogenic (low) temperature of 79 K \( \pm 1 \text{ K} \).

In phototrophic bacteria such as *Rba. sphaeroides* the peripheral LH2 complexes donate solar excitation energy to the core LH1 complexes, which encircle the RCs, and from there to the RCs (see [16, 17] for reviews). In the RCs the excitation energy is then transformed into potential chemical energy. The WT *Rba. sphaeroides* RC is made up from three membrane-spanning polypeptides, two of which (named L and M) possess very similar tertiary structures with five membrane-spanning \( \alpha \)-helices linked by a series of small helices and loop regions. Together, the membrane-spanning \( \alpha \)-helices of L and M form a protective scaffold for the non-covalently bound cofactors: six bacteriochlorin chromophores (four BCHls and two bacteriopheophytins (BPhes)), two quinones, one carotenoid and one non-heme iron (figure 1(A)). The bacteriochlorin molecules and quinones are arranged in pairs around a pseudo-C\(_2\) symmetry axis which runs from the center of the primary donor of electrons (a pair of closely interacting BCHls, also called special pair, hereafter denoted as P) through the two accessory BCHls (collectively denoted as B) and two BPhes (H) to the non-heme iron [18]. The individual cofactors in the bacterial RC are thus distributed throughout the protein volume, setting up a network of optical probes for the monitoring of any structural changes that occur in response to external stress through optical spectroscopy.

In the WT RC just one of the \( \pi \)-conjugated carbonyl groups out of the four available in the two P BCHls is occupied by H-bond (at the C\(_3\) position of the P\(_L\) BCHl, see figure 1(B)). This violation of the apparent C\(_2\) symmetry may be one of the reasons that the energy transduction and electron transfer within the RC is highly asymmetric, taking primarily place along the branch of chromophores closely associated with the L subunit (left branch in figure 1(A)).

**Figure 1.** Architecture of the cofactors of the *Rba. sphaeroides* RC. (A) Overall cofactor arrangement around an axis of two-fold symmetry. (B) The special pair of BCHl molecules viewed along the symmetry axis. Histidine L168 donates a H-bond to the acetyl carbonyl oxygen of P\(_L\) (magenta dashes). The symmetrical residue is a non-bonding phenylalanine.

### 2. Materials and methods

#### 2.1. Materials

The samples under study were prepared from the antenna-deficient *Rba. sphaeroides* strain DD13, as described previously [19]. After breakage of harvested *Rba. sphaeroides* cells, photosynthetic membranes containing just RC complexes and devoid of any LH complexes (further denoted as m-RC) were isolated by ultracentrifugation on 15%/40% (w/v) sucrose gradients and concentrated by ultracentrifugation onto a 60% (w/v) sucrose cushion, followed by dialysis to remove excess sucrose. Purified RC complexes (i-RC) were prepared by nickel affinity chromatography [20]. Samples of concentrated membrane or protein stored at liquid nitrogen temperature were thawed before the experiments and diluted with 10–20 mM TRIS, pH 7.5 to obtain an optical density of about 0.3 at 800 nm in the assembled pressure cell. The buffering ability of TRIS is known to be preserved over a
Figure 2. Schematic view (not to scale) of the bubble-free immersion cryostat for optical studies of the samples compressed in a high-pressure cell. 1-high pressure cell, 2-piston, 3-high pressure cell flange, 4-nut for fixing the cell, 5-He capillary, 6-sample, 7-sapphire windows, 8-vacuum jacket of the cryostat, 9-pipelet for evacuating the vacuum jacket, 10-viton gasket, 11-thermal insulator, 12-LN$_2$ evaporator, 13-manometre, 14-manostat, 15-pipelet for filling the main vessel of the cryostat with LN$_2$, 16-pipelet for filling the evaporator with LN$_2$, 17-optical windows.

broad temperature and pressure range [21, 22]. The buffer for i-RCs additionally contained 0.03% lauryldimethylamine-N-oxide (LDAO). In addition, 5 mM sodium ascorbate and 25 $\mu$M phenazine methosulfate was present in the buffer solution to prevent oxidation of the special pair by the measuring light. Addition of glycerol to the buffer with a 2:1 volume ratio secured transparent glassy samples for low temperature measurements.

2.2. High-pressure barospectroscopy

The piston-cylinder type high-pressure optical cell with sapphire windows used in this work has been described previously [23, 24]. It involved a separate sample cell of $\sim$0.3 ml volume formed by 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 three-stage compressor and was transmitted to the sample by helium gas. A manganin-wire gauge was used to measure pressure in the cell and calibrate the equipment. Samples were allowed to equilibrate for 10–15 min after an increase in the pressure. Reversibility of the pressure-induced effects was checked systematically by measuring the spectra upon pressure release within $\sim$15 min, after the pressure maximum had been applied. Experimental uncertainty for pressure was $\pm$10–20 MPa, depending on the measurement.

A special bubble-free liquid nitrogen immersion cryostat shown in figure 2 was developed for high-pressure experiments at liquid nitrogen (LN$_2$) temperatures [25]. Main parts of the cryostat were a hermetically closed manostated cryovessel that encloses the high-pressure cell and the evaporator. The evaporator adsors the heat flux from the high-pressure cell and other parts of the cryostat during its operation. Inside the evaporator, which is open to the atmosphere, liquid nitrogen is boiling at ambient pressure. The liquid nitrogen surrounding the high-pressure cell is prevented from boiling due to a slight overpressure in the cryovessel. In our practice, about 1/3 of the evaporator was submerged in the LN$_2$ of the main cryovessel, the overpressure was 80–100 Torr controlled by the manostate and visualized by the manometer. The temperature of the LN$_2$ in the main cryovessel was 2 K higher than in the evaporator. The evaporator had to be periodically refilled with liquid nitrogen, whereas its level in the main cryovessel remained unchanged for the whole experimental time.

Absorption/transmission spectra of the samples were recorded with one-beam measuring system based on a MDR-23 spectrometer (LOMO, Russia), a CCD camera (DUS DV420A-OE, Andor Technology) and a stabilized tungsten incandescent lamp. The light of the lamp was directed through the sample in high pressure cell and then focused to the entrance slit of the spectrometer. Spectral resolution of the system was 0.3 nm. Spectra were corrected by subtracting, at each applied pressure, a reference spectrum corresponding to that of the detergent-containing buffer alone in the pressure cell. Fluorescence spectra were measured on the same system using a Coherent 700 mode-locked dye laser synchronously pumped at 76 MHz by a Coherent Antares 76 s Nd:YAG laser for excitation. To diminish the influence of the scattered excitation laser light on the measured spectra the sample in high pressure cell was excited in reflection geometry employing additional laser cut-off filter. Several measurements were carried out for every sample to ensure reproducibility of the data.

2.3. Data analysis

Prior to data analysis the absorption spectra were converted from nanometers to an energy scale (wavenumbers, cm$^{-1}$). The main absorption band parameters, position and width, were determined by means of curve fitting algorithms available in Origin (Microcal Software, Inc.). The estimated accuracy of the band positions and widths depended on the signal-to-noise ratio, and was between 0.2 and 1.5 nm.

3. Results and discussion

3.1. Pressure-dependent absorption spectra

3.1.1. Physiological temperatures. At ambient pressure and temperature, the bacteriochlorin cofactors described in figure 1 are pairwise distinguishable in the near-infrared spectral range (figure 3) with the $Q_y$ absorption bands at around 870, 800 and 760 nm attributable, respectively, to the special pair BChls (P), the two accessory BChls (B) and the two BPhes (H). Upon compression all three $Q_y$ absorption
The absorption spectrum of isolated reaction center complexes observed in other chromoprotein complexes are generally not linear. This is a common behavior, also discussed in more detail below, the shifts plotted on an energy preserving the overall shape of the spectrum. As will be bands consistently shifted towards red
(longer wavelengths) preserving the overall shape of the spectrum. As will be discussed in more detail below, the shifts plotted on an energy scale are generally not linear. This is a common behavior, also observed in other chromoprotein complexes [2–5, 23, 26, 27]. We further notice that the initial (low-pressure) shift rate of the P band was significantly greater compared with the rates of shift of either the H or B bands, which were rather similar to one another (see Table 1 for more details). This corroborates with the generally accepted different physical origins of these bands, excitonic in the case of P [28, 29] and quasi-monomeric in the cases of both H and B [29, 30], see [6] for a review.

Another major observation, more clearly demonstrated in Figure 4, was that there was little difference in the conduct of B and H bands in i- and m-type samples at all pressures. The bands, excitonic in the case of P and quasi-monomeric in the cases of both H and B [29, 30], see [6] for a review.

**Figure 3.** Near-infrared absorption spectra of the detergent-isolated RC complexes recorded at ambient temperature of 295 K (left panel) and low temperature of 79 K (right panel). Spectra normalized according to the maximum of the B band at about 800 nm are displaced vertically relative to one other proportional to the experimental pressure indicated at each curve. Dotted lines follow peak positions of the P, B, and H absorption bands at different pressures. Two bottom contours represent initial (solid line) and final (dashed line, measured after pressure release) spectra. The almost perfect overlap of the spectra indicates that pressure effects were reversible both at physiological and cryogenic temperatures.

**Table 1.** Linear pressure-induced shift rates of P, B, and H bands in the absorption spectrum of isolated reaction center complexes.

<table>
<thead>
<tr>
<th>Transition</th>
<th>Temperature, K</th>
<th>-134.4 cm⁻¹/100 MPa</th>
<th>-70.1 cm⁻¹/100 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>295 K</td>
<td>BLM</td>
<td>BM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-30.5 cm⁻¹/100 MPa</td>
<td>-12.9 cm⁻¹/100 MPa</td>
</tr>
<tr>
<td>H</td>
<td>79 K</td>
<td>-39.3 cm⁻¹/100 MPa</td>
<td>-16.5 cm⁻¹/100 MPa</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>-20.3 cm⁻¹/100 MPa</td>
<td></td>
</tr>
</tbody>
</table>

* Determined from a second-order polynomial fit of experimental dependences.

* Standard deviation associated with several independent measurements is ±7%.

**Figure 4.** Pressure dependences of the transition energy and width in the absorption spectra of detergent-isolated (empty symbols) and membrane-embedded (solid symbols) RC complexes at two different temperatures: 295 K (left panels) and 79 K (right panels). Band positions are presented for the P (A), B (C) and H (D) bands, while band widths (HWHM at high temperature and FWHM at low temperature, see text for further explanation) only for the P band (B). Energy scale in panels C and D is twice expanded compared with the scale in panel A. Red lines represent second-order polynomial fits of the experimental dependences.

larger than expected H-band position difference (Figure 4(D)) is a probable result of the interference between the H absorption band and the band of solubilized BCHls, which in various quantities always presents in detergent-isolated preparations. At the same time, the P band trajectories demonstrated in figure 4(A) were similar only at rather low pressures, below about 200 MPa. At higher pressures, the spectral shifts for m- and i-RCs strongly deviated from one another. While in m-RCs the red-shift was continuous, albeit with decreasing pace with increasing pressure, in i-RCs the red-shift stopped or even reversed at a medium pressure range (200–350 MPa) before continuing to red-shift at still higher pressures.

This deviating behavior repeated itself in the band width dependences (Figure 4(B)). While for m-RC complexes the width was almost constant over the whole pressure range, for i-RC complexes it was constant only up to about 200 MPa, abruptly broadening afterwards. It is worth noting here that due to the considerable overlap of the P and B bands at ambient temperature that further increases with pressure (see figure 3(A)), the high-temperature width of the P band was defined as the half width at half maximum (HWHM),
determined from the long wavelength side of the band. Generally, this side could be well described by a Gaussian shape. At low temperature the P band separates well from the rest of the bands, allowing the usual full width at half maximum (FWHM) definition to be utilized.

The dependences observed are in fair agreement with previous measurements [12, 13]. By combined electronic absorption and Fourier-transform pre-resonance Raman spectroscopy it was established that the detergent-isolated WT RC and a carotenoid deficient variant did not lose their three-dimensional structures up to 600 MPa [12, 13]. Nevertheless, a number of local reorganizations in the binding site of the primary electron donor were observed in Raman studies conducted between atmospheric pressure and 200 MPa. Although no more structural reorganization of this binding site could be observed by Raman spectroscopy, the absorption spectra showed, like in figures 3 and 4(A)/(B), that the electronic structure of P became dramatically perturbed past about 200 MPa.

The cause of this perturbation remained open. However, our subsequent studies by comparing the pressure-dependent spectra of WT (one H-bond) and HL168F mutant (zero H-bonds) RCs convincingly established [15] that this effect is due to the pressure-induced rupture of the lone H-bond between the acetyl carbonyl side-group of the P$_L$ BChl molecule and the histidine residue of the surrounding protein scaffold (figure 1(B)). At ambient pressure and temperature, the brakeage of the H-bond results in a 27° through-plane rotation of the acetyl carbonyl of the P$_L$ BChl with respect to orientation of this side-group in the WT RC complex [31]. Such rotation is known [32] to yield a blue spectral shift, by shrinking the size of the excited state wave function of the P$_L$ BChl chromophore. Similar interpretation for spectral shifts resulting H-bond breakage in bacterial LH complexes was provided in [33–35].

3.1.2. Cryogenic temperatures. The low-temperature absorption spectrum of detergent-isolated RC complexes in dependence on pressure is overviewed in right hand side of figure 3, and detailed in right hand side of figure 4. With lowering the temperature the special pair band rapidly shifted towards red and narrowed, so that the P band became well separated from the rest of the spectrum. The apparently homogeneous at ambient temperatures H and B bands split without much shifting, revealing their H$_L$/H$_M$ and B$_L$/B$_M$ sub-components. Since P, the lowest-energy exciton band of the special pair, is most definitely homogeneously broadened, it did not divide upon cooling. These well-known thermal effects have been reviewed [6].

Upon compression, all the bands yet again demonstrated a red shift. However, the shift trajectories and rates observed at low temperatures were very different from those at high temperature. Specifically, as demonstrated in table 1, the linear pressure-induced shift rates evaluated in the zero-pressure limit at low temperature appeared considerably, about twice smaller than those at high temperature. Similar trend was previously observed in LH1 and LH2 protein complexes [23, 36]. Such significant drop of the shift rate at low temperature was in [36] explained by decreasing compressibility of the proteins upon freezing. The present data thus generalizes this result for another type (RC) of membrane protein complexes.

3.2. Validation of the absorption band shifts at physiological and cryogenic temperatures

Presented in figure 5 is the relative absorption band shifts of isolated RC complexes recorded at high and low temperatures. These pressure dependences evaluated from the absolute band position data of figure 4 rather well illustrates the many differences existing between the different RC bands as well as between the measurements performed at various temperatures (more generally, at various thermodynamic conditions). Data of table 1 add quantitative basis to this qualitative consideration.

Figure 5 once again confirms the qualitatively different response on pressure of the P band at high and low temperature. The observed at low temperature monotonous and quasi-linear pressure shift together with the constant bandwidth suggest little if any structural changes in the vicinity of the special pair over the experimental pressure range. We will discuss the high-temperature performance of the P band in some detail in section 3.4.

Similarly great at all temperatures are differences in sensitivity and character of the P band shift from that of the B or H bands. Figure 5 additionally brings out variances, both at high and low temperatures, between the behavior of B and H bands, which were not so obvious in figure 4. Furthermore, the low-temperature data detail that the shift rates and shift character for the separate L and M components of B and H slightly differ from each other. Taken together, this rich selection of variances emphasizes the importance of local environment in the observed phenomena.
3.3. Comparison of absorption and fluorescence spectra of the special pair

Due to photo-chemically induced quenching of the excited special pair in picosecond time scale, the fluorescence of RCs at normal conditions is weak (figure 6) and difficult to measure on the background of the fluorescence of solubilized, non-functional BChl molecules, which in small quantities always present in membrane protein preparations [37, 38]. This interference, which in practice affects most the determination of the band widths, is in certain degree relaxed at low temperatures where the solubilized and protein-bound BChl fluorescence bands are spectrally more separated. High-pressure compression of the samples resulted in further weakening of the emission.

Figure 7 compares pressure dependences of the special pair absorption and fluorescence band positions (A) and bandwidths (B) as well as of Stokes shift (C) between the absorption and fluorescence spectra for detergent-isolated RC complexes at high and low temperatures. The situation at low temperature, as demonstrated on right hand side panels with practically parallel shift (A) of absorption and fluorescence spectra, and constant width and Stokes shift, appears straightforward. This is what one would expect to observe in the case of one and the same (resonant) molecular electronic transition active in absorption as well as in fluorescence. The considerable width of the special pair band is explained by strong exciton–phonon coupling due to charge transfer character of the P transition [6, 28, 29]. Assuming, as usual, that the absorption and fluorescence spectra of the special pair are homogeneously broadened at cryogenic temperatures, their different bandwidth suggests lack of mirror symmetry of the spectral transition under consideration [39].

The situation at high-temperature appears much more complex (figure 7, left side). The absorption and fluorescence peaks (A) follow parallel courses only up to about 200 MPa, the established beginning of the H-bond rupture, splitting off afterwards. Correlated variations of width (B) and Stokes shift (C) dependences corroborate this observation. Approximate levelling off of the Stokes shift past about 400 MPa indicates that at this pressure range the traces representing absorption and fluorescence once again run in parallel.

The significantly greater width and Stokes shift recorded at ambient temperature compared with these at low temperature is also worth noting. They most probably reflect the increased conformational flexibility of the protein at elevated temperatures. The fluorescence spectrum at ambient pressure and temperature appeared broader than the concomitant absorption spectrum. Yet this order, similar to the low-temperature observation, was at high temperature reversed at about 400 MPa. This is because the specific broadening of the absorption bandwidth, as was already described in relation to figure 4(B), while the fluorescence bandwidth almost did not change with pressure.

The latter behavior still awaits explanation. One way to understand this is by assigning different emission properties for the special pair exciton states with H-bonds intact or broken, and assuming that only the H-bonded special pairs fluoresce significantly. This hypothesis would qualitatively explain not only the smaller (compared with absorption) irregularity of the fluorescence band position and its almost constant width but also the noted decrease of the fluorescence intensity under compression. We are going to pursue these interesting correlations in a forthcoming study.
compressed state with broken H-bond(s). The equilibrium constant of such a two-state denaturation reaction (which likens a phase transition) may be written as:

$$K(P) = [D]/[N] = \exp[-\Delta G(P)/RT]. \quad (1)$$

Here, [N] and [D] indicate the concentrations of native and denatured proteins, respectively, $R$ is the universal gas constant, $T$ is the thermodynamic temperature, $P$ is the pressure, and $\Delta G$ is the free energy change associated with the protein denaturation. In linear approximation:

$$\Delta G(P) = \Delta G^0 + \Delta V^0 P,$$  \quad (2)

where $\Delta G^0 = G^0_N - G^0_N$ is the standard free energy difference between the denatured and the native states, and $\Delta V^0 = V_D - V_N$ is the standard partial molar volume change between the states.

A connection of this model with spectroscopic experiment is established by calculating the pressure-dependent equilibrium constant as:

$$K(P) = [\Delta \nu(P) - \Delta \nu_i]/[\Delta \nu_f - \Delta \nu_i], \quad (3)$$

where $\Delta \nu_i(P)$ is the relative peak shift at pressure $P$, and $\Delta \nu_i$ and $\Delta \nu_f$ are the shifts measured at initial (i) and saturating final (f) pressures, respectively. It then follows that

$$-RT \ln K(P) = \Delta G^0 + \Delta V^0 P.$$  \quad (4)

The solution of equation (4) provides prime model parameters, $\Delta V^0$ and $\Delta G^0$, as the slope and initial ($P = 0$) value, respectively.

Application of the above model for the evaluation of the H-bond energy (formally defined as the H-bond rupture free energy difference $\Delta G^0$) is shown in figure 8(B). As can be seen, the slope (i.e. $\Delta V^0$) is negative, meaning that the denatured state has smaller volume compared with the native state. The denatured state must thus become stabilized upon the volume compression, as is confirmed by the experiment. Parameters drawn out from this simple analysis are as follows: $\Delta G^0 = 12.0 \pm 1.2$ kJ mol$^{-1}$, $P_{1/2} = 265 \pm 27$ MPa, $\Delta V^0 = -46 \pm 5$ ml mol$^{-1}$.

### 4. Summary and concluding remarks

The assembly and function of complex structures in nature is driven by a multitude of intermolecular interactions, notably that of H-bonds. Understanding and ultimately controlling the functioning of biomaterials requires extensive study of how these forces drive local nanoscale structures and interactions. In this work, the effects of application of high external pressure on the structure of the WT reaction center from *Rba. sphaeroides* was studied. Our results indicate localized differences in protein compressibility both at ambient and cryogenic temperatures when using the intrinsic chromophores as molecular probes. Different behavior of absorption and fluorescence spectra suggests that the fluorescence intensity loss is induced by a slight change of the primary electron donor structure, allowed by the lone H-bond to the active-side BChl chromophore. The individual H-bond
strength in the detergent isolated WT RC, defined as the free energy change associated with the rupture of the bond, was estimated to be equal to 12 kJ mol\(^{-1}\), equivalent to 1000 cm\(^{-1}\) or 0.124 eV. In molecular terms, this is a relatively strong bond corresponding to about 5 kBT. Comparable values were previously found for the LH membrane chromoproteins of purple bacteria [4–7]. The significant role of the native membrane in stabilization of the membrane protein structure is revealed by different rupture pressures observed in case of i- and m-type RC complexes, being about 265 MPa for detergent-isolated complexes and 500 MPa for membrane-protected complexes. Although the specific mechanism of the H-bond rupture was not studied in this work, our data corroborate a mechanism for the pressure-induced destabilization of the protein structure in which water penetration into the hydrophobic interior plays central role [43, 44].

Acknowledgments

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