Chemical Physics 490 (2017) 81–91

Contents lists available at ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

Stability and properties of quasi-stable conformational states in the LH2 light-harvesting complex of *Rbl. acidophilus* bacteria formed by hexacoordination of bacteriochlorophyll *a* magnesium atom

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ARTICLE INFO

Article history:
Received 7 November 2016
In final form 14 April 2017
Available online 20 April 2017

Keywords:
Light harvesting complex
Purple bacteria
LH2
Conformational changes
Magnesium ligation

ABSTRACT

Single-molecule spectroscopic experiments on several light-harvesting complexes revealed the existence of a set of metastable conformational states with different spectroscopic properties and lifetimes spanning from milliseconds to tens of seconds. In the absence of explicit structural data, a number of probable structural changes underlying the observed spectroscopic shifts were proposed. We examine the donor-acceptor interaction between the magnesium atom and the acetyl group of the adjacent bacteriochlorophylls *a* as a possible origin of metastable conformational states in the LH2 light-harvesting complex of *Rbl. acidophilus* bacteria. The results of QM/MM and molecular dynamics simulations show that such ligation can occur at room temperature and leads to one metastable coordination bond per pair of bacteriochlorophylls in the B850 ring. According to the results of Poisson-TrESP modeling, such coordination lowers the energies of the excited states of the complex by up to 163 cm^{-1} which causes red spectral shift of the B850 band.

1. Introduction

Single molecule spectroscopy (SMS) has proved to be a powerfool tool for examining the surroundings of the luminescent molecules and for probing the surfaces of the solids [1,2]. The absence of ensemble averaging in this spectrosopic method allows one to obtain a detailed information about the inhomogeneity of an environment of the spectroscopically active molecules and the interaction between them. Application of SMS to several light-harvesting complexes, namely LH2 complexes of purple bacteria *Rbl. acidophilus* [3–16],*Rh. sphaeroides* [10] and *Rs. molischianum* [17,10], LH1 complex of *Rh. sphaeroides* [10] and light-harvesting complexes of cyanobacteria [18] and plants [19–22], revealed the so-called spectral jumps, or variations with time of fluorescence wavelength and intensity of the pigment molecules ensemble within the complex with the magnitudes of tens to hundreds of cm^{-1}. These variations can be interpreted as the transitions of the light-harvesting complex between several states which differ by excitation energies and fluorescence quantum yields. Transitions between these states are reversible and can occur both under illumination [5,6,9,10,14,15,19–22] (even at cryogenic temperatures [3,4,7,8,17,18,16]) and in the dark [11]. In the latter case, the characteristic times of transition are of the order of tens of seconds [11,13] while in the former case the rates of transitions are proportional to the illumination intensity [13].

The nature of the states with different spectroscopic properties can not be revealed directly from spectroscopic data (and is not necessarily the same for different complexes), and several hypotheses were proposed, including the formation of the long-lived charge-separated states [22], coordination of Mg^{2+} ions by protein [12] and the existence of several metastable conformational states of the pigment-protein complex [23–25]. In the case of LH2 complexes of purple bacteria, and, specifically, of *Rbl. acidophilus*, which is in the focus of this paper, the latter hypothesis is the most widely supported [23], given that the transitions are reversible and occur in different media and at different temperatures, are pH-inducible for some complexes [25], and their times far exceed the lifetimes of singlet (~1 ns [26,27]) and triplet (~7 μs [28]) electronic states of the isolated complex.

The structure of the LH2 complex of *Rbl. acidophila* (previously denoted as *Rps. acidophila* [29]) was determined by X-ray crystallography [30–32]. This ring-like complex has C_9 symmetry and comprises 9 α,β-heterodimers of protein molecules connected to the pigments: a pair of closely located bacteriochlorophylls *a* (BCLs), one more BCL and a carotenoid, rhodopin glucoside (RG). Due to the symmetry of the complex BCLs form two rings: B850 ring, consisting of 18 closely packed BCLs, and a more sparce

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http://dx.doi.org/10.1016/j.chemphys.2017.04.010
0301-0104/

B800 ring (see Fig. 1). Due to the differences in protein surroundings and pigment-pigment interactions these two rings give rise to two separate bands in the absorption spectrum with the maxima near 800 and 850 nm (hence the names of the rings). The interaction of the BCLs between and within the rings leads to rapid excitation energy transfer on a sub- and picosecond scale within the complex [33–35]. Similar structures, with C_9 and C_8 symmetries, respectively, were found for the LH2 complexes of *Rh. sphaeroides* [36] and *Rs. molischianum* [37].

Since single-molecule spectroscopy does not provide explicit structural information, several models of structural alterations of LH2 antennae accounting for the observed spectral shifts were proposed. The early hypothesis supported by the X-ray scattering experiments and SMS in combination with theoretical modeling attributed the phenomenon to spontaneous elastic elliptic deformations of the LH2 ring which lower the symmetry of the ring from $C_9(C_8)$ to C_2 pseudosymmetry [6,4,8,39,40]. These deformations affect the structure of the band of excitonic states which leads to elimination of excited states' degeneracies, to changes in states' energies and in the oscillator strength values corresponding to the excited states ground state transitions. All those changes manifest themselves as changes in the fluorescence wavelengths and quantum yields.

Later it was found that there must be some additional local structural changes of BCL environment to account for all spectral details. Novoderezhkin et al. proposed a general model for the LH2 antenna which includes elliptic deformations of the entire complex combined with local disordering [41]. Using this model, different regimes of excited-state dynamics driven by interaction of excitons with fast and slow vibrational modes were found, namely coherent exciton motion and incoherent hopping (which might result in trapping of excitation by a group of 3–4 BCLs), which correspond to different spectral features. Valkunas et al. showed that the existence of two distinct minima on the potential energy surface (PES) both in ground and first excited electronic states of each BCL in the B850 ring makes it possible to explain the fact that spectral jumps occur under illumination and in the dark and to reproduce the fluorescence statistics [13,42,43]. A similar model, with four conformational states per a pair of BCLs, was used by Novoderezhkin to model the conformational dynamics and spectral statistics in the LH2 complex of *Rbl. acidophila* [14]. Later an analysis of excitation lifetime fluorescence intensity diagrams obtained by SMS of LH2 antenna of *Rbl. acidophila* in solution revealed the existence of three distinct states of the complex (one of them having two substates) which are formed due to conformational changes and photobleaching of the pigments [15]. In a later study of the LH2 complexes in a polymer film it was found that the same pigment-protein supramolecule can have different number (namely, two, three or four) of conformational states [44,45].

In this paper, we examine the structural changes in the LH2 complex of *Rbl. acidophila* underlying the fluorescence spectral shifts of a B850 ring revealed by SMS. In the first part, we analyze the driving forces of the structural deformations of the complex using CASSCF calculations for BCL molecule and propose a model for such deformations. In this model, we suppose that coordination of an acetyl group of a BCL by magnesium atom of an adjacent BCL is responsible for the observed spectroscopic changes. In the second part, we use the QM/MM method to verify that such coordination is possible and calculate the spectral properties of the coordinated and non-coordinated conformations by means of Poisson-TrESP approach [46]. In the third part, we discuss the results of molecular dynamic simulation of the LH2 complex which show that the transitons between the coordinated and non-coordinated conformations can be caused by thermal activation.

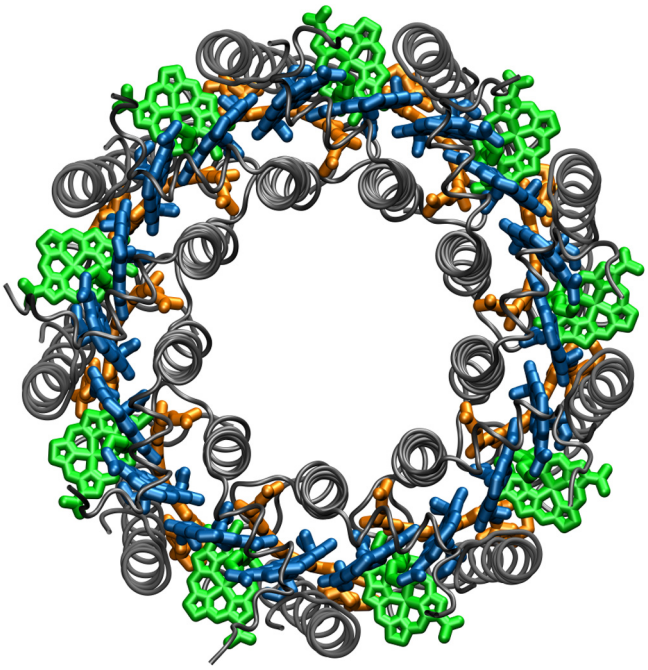

Fig. 1. The structure of the LH2 antenna of Rbl. acidophila. Protein is shown in grey, carotenoid molecules are shown in orange. Bateriochlorophylls *a* of the B800 and the B850 rings are shown in green and blue, respectively. The image is produced using the structural data from RCSB data bank (entry 2FKW [32]) and the VMD program [38].

2. Computational details

Structural data from RCSB (entry 2FKW [32]) was used for calculations involving either an entire LH2 complex or its constituents.

2.1. Individual BCLs

For calculations of properties of individual BCL molecules in the ground and the first excited electronic states, CASSCF method and 6-31G(d) basis were used. FIREFLY [47] program (partially based on GAMESS US source code [48]) was utilized for these calculations. The nonpolar phytol residue of the BCL was replaced by methyl to reduce the computational cost. Four orbitals (HOMO−1 to LUMO+1) were included into the active space.

Geometry optimizations and hessian calculations were performed for the S_0 and S_1 states with the aid of state-specific CASSCF. In addition to hessian calculations, the potential energy surface was scanned along the dihedral angle which corresponds to rotation of an acetyl group of BCL. This was done as follows: first, a ground state relaxed scan on CASSCF/6-31G(d) level was carried out; second, the state-average CASSCF energy calculation was performed for each point on the scan; third, a 2nd order perturbation theory correction to CASSCF results (in XMCQDPT variant, as implemented in the FIREFLY program [49]) was utilized to account fully for the effects which are produced on the electronic system of BCL by acetyl rotation.

To determine excitonic couplings in the LH2 complex, ESP and TrESP [50] atomic charges were calculated for the BCL molecule. State-average (equal weight) CASSCF calculation was performed for BCL in the ground state equlibrium geometry to obtain the wave functions. Those were used to calculate the values of the molecular electrostatic potential in the vertices of a geodesic grid [51] which span from 6 to 12 Å from the atoms. The ESP charges