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Structural model and excitonic properties of the dimeric RC–LH1–PufX complex from *Rhodobacter sphaeroides*

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ABSTRACT

The light-harvesting apparatus of the purple bacterial photosynthetic unit consists of a pool of peripheral light-harvesting complexes that transfer excitation energy to a reaction center (RC) via the surrounding pigment–protein complex LH1. Recent electron microscopy and atomic force microscopy studies have revealed that RC–LH1 units of *Rhodobacter (Rba.) sphaeroides* form membrane-bending dimeric complexes together with the polypeptide PufX. We present a structural model for these RC–LH1–PufX dimeric complexes constructed using the molecular dynamics flexible fitting method based on an EM density map. The arrangement of the LH1 BChls displays a distortion near the proposed location of the PufX polypeptide. The resulting atomic model for BChl arrays is used to compute the excitonic properties of the dimeric RC–LH1 complex. A comparison is presented between the structural and excitonic features of the S-shaped dimeric BChl array of *Rba. sphaeroides* and the circular BChl arrangement found in other purple bacteria.

1. Introduction

Photosynthetic light harvesting is achieved by the cooperation of up to hundreds of proteins containing thousands of pigments capturing and transferring light energy for subsequent conversion to stable chemical energy [1–6]. This conversion is initiated at a reaction center (RC) that utilizes the electronic excitations received from the surrounding pigment pool for transmembrane electron transfer (for reviews see Refs. [7–14]). The charge gradient thus created is converted to chemical energy in either oxygenic or anoxygenic photosynthetic processes, the latter being governed by evolutionarily more primitive systems [15]. The anoxygenic purple bacterial photosynthetic unit (PSU) constitutes a remarkably simpler light-harvesting system than those found in cyanobacteria and plants. The atomic structure of purple bacterial RCs [16–20] and their surrounding light-harvesting antennae [21–27] permitted the elucidation of the light-harvesting process down to a quantum mechanical level [28–49,9,50]. Furthermore, the supramolecular organization of the participating photosynthetic proteins has been investigated in experimental [51–56,20,57–59] and theoretical [60–63,50] studies.

The purple bacterial PSU from *Rhodobacter (Rba.) sphaeroides* consists mainly of a peripheral pool of light-harvesting complex II (LH2) units that surrounds arrays of reaction center–light-harvesting complex I–PufX (RC–LH1–PufX) dimers which induce membrane curvature [64]. The dimeric architecture of RC–LH1–PufX units of *Rba. sphaeroides* [59] is not realized in other purple bacteria, such as *Rhodospirillum rubrum* [52], that involve only monomeric RC–LH1 complexes. Further constituents of the PSU are the bc_1 complex [65–68], cytochrome c_2, and ATP synthase [69–71], which jointly convert the charge gradient, created after light harvesting by the LH2 and RC–LH1–PufX units, into stable chemical energy in the form of ATP.

The PSU is spatially organized into lamellar or spherical pseudo-organelles. The respective membrane curvature induced by the constituent pigment–protein complexes of the PSU is essential for photosynthetic function. A recent molecular dynamics (MD) study of the photosynthetic membranes in *Rba. sphaeroides* revealed that intrinsic curvature is spontaneously induced by an array of membrane-embedded LH2 units, whereas an initially flat RC–LH1–PufX dimer spontaneously bends at the dimerization junction [64]. Single-particle electron microscopy (EM) analysis of negatively stained RC–LH1–PufX dimeric complexes in *Rba. sphaeroides* displayed bending between RC–LH1 monomers [59]. The curvature

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of the photosynthetic membrane induced by these pigment–protein complexes increases the effective surface area per unit volume available for light harvesting and likely optimizes the electrostatic and chemiosmotic potentials governing energy conversion as suggested recently in view of the observed curvature around ATP synthase dimers [72]. As a result of the induced curvature, the PSU of *Rba. sphaeroides* forms pseudo-spherical invaginations of the inner membrane with a diameter of approximately 70 nm [50]. The resulting volume of the vesicle ensures the necessary cytochrome c_2 concentration to reduce the RCs and the proton concentration to drive the ATP synthase.

Recent coarse-grained Monte Carlo studies modeled the spontaneous generation of photosynthetic vesicles due to the accumulation of curvature by its constituents [73]. An atomic level description of such a photosynthetic vesicle for *Rba. sphaeroides* was presented in [50], based on atomic force microscopy (AFM) [53,54,56,20,58], cryo-EM [51,52,55,57], and linear dichroism [56] data as well as on available atomic structures of the constituent proteins. This description elucidated the quantum mechanical basis of efficient light harvesting across an entire photosynthetic membrane entailing about 200 proteins and 4000 bacteriochlorophylls (BChls) [50].

The aforementioned theoretical studies [60–63,50,73,64] constitute the first steps toward a comprehensive biology of the PSU by combining spectroscopy, structure, and energy transfer dynamics data with a multitude of simulation techniques, bridging length scales of 10^0–10^4 Å (atomic to vesicular sizes) and time scales of 10^{-15}–10^{-3} s (inter-pigment excitation hopping to vesicle curvature formation times).

Accurate vesicle-scale structural models of the PSU require the availability of the atomic structures of the constituent molecules. Unfortunately, no atomic structure is currently available for the membrane-bending dimeric S-shaped RC–LH1–PufX complex of *Rba. sphaeroides*. In an earlier study of *Rba. sphaeroides* vesicles [50] a planar model of the dimeric RC–LH1–PufX BChl array was constructed based on single-particle EM analysis [57] available at the time. However, recent data collected from electron micrographs of over 4000 negatively stained dimer particles enabled the reconstruction of a 3D map of the dimer complex at 25 Å resolution. This map [59] indicates that the RC–LH1–PufX dimer exhibits a bending angle of 146° at the dimerization junction.

In the current study as well as in a complimentary study [74], we present an atomic structural model for the *Rba. sphaeroides* RC–LH1–PufX complex based on single-particle EM analysis [59]. The structural model is obtained using the molecular dynamics flexible fitting (MDFF) method [75] already successfully applied to the ribosome, but under vacuum conditions [76]. The MDFF methodology combines in the present case the MD simulation of an earlier planar model of the RC–LH1–PufX complex [50,64] in a membrane-water environment with the observed 3D cryo-EM map [59], introducing the latter into the MD force field [75]. The resulting all-atom model not only elucidates the structural basis of the membrane-bending curvature of the complex, but also provides information on the spatial arrangement of the BChl array of RC–LH1–PufX dimers. The geometry of the BChl array thus established permits on the one hand the calculation of the excitonic properties of the complex following the effective Hamiltonian approach [37–40,77,45,42–44,78,79,48,49,9,80,81,50] and on the other hand description of RC–LH1–PufX dimers forming vesicles and tubes [74]. Altogether the broad purpose of the current study is then twofold: first, to explore the geometry of the BChl array in the dimeric RC–LH1–PufX complex; second, to determine computationally the ramifications of this geometry for the quantum biological characteristics of the system.

The organization of this paper is as follows. First, the MDFF protocol employed for the generation of the atomic structural model for the RC–LH1–PufX dimer is described in detail. Then the effective Hamiltonian formulation for the BChls of the RC–LH1–PufX dimer is introduced as the basis of the excitonic structure computations for the BChl array obtained by the MDFF study and applied to determine the spectral properties and excitation transfer dynamics of the complex.

2. Methods

2.1. Molecular dynamics flexible fitting of the RC–LH1–PufX dimer

MDFF is a modeling technique which uses a MD simulation to flexibly fit atomic structures into electron microscopy maps [75]. In an MDFF simulation, two external potentials (U_{EM}, U_{SS}) are applied to the system in addition to the usual MD potential (U_{MD}). Thus, the total energy of the simulated system is

$$U_{total} = U_{MD} + U_{EM} + U_{SS}, \quad (1)$$

where U_{MD} refers to the molecular dynamics force field, U_{EM} is derived from the EM density map to steer the atoms toward filling the regions with high observed density, and U_{SS} is a harmonic potential that preserves the secondary structure to prevent overfitting.

The MDFF method requires a starting atomic model for the constituents that are being fitted to the target EM density map. A planar atomic model for the RC–LH1–PufX dimer of *Rba. sphaeroides* was provided in [64] based on available solution structures and 2D EM projection data [57]. As described in [64], an NMR solution structure (PDB id: 1DX7) [25] was used for the LH1 β-apoprotein. For the LH1 α-apoprotein a homology model was built in the absence of an available structure based on the *Rhodospirillum rubrum* LH1 α-apoprotein reported in [82]. The bacteriochlorophyll and carotenoid molecules were then placed in between the α/β-apoprotein pair to form an LH1 subunit, their positions chosen to be similar to those in *Rhodospirillum molischianum* LH2 [24]. This step was followed by energy minimization to ensure proper ligation. The LH1 α/β subunit was then replicated into 28 copies and arranged into an S-shaped dimer based on the cryo-EM data reported in [57]. Two RCs (PDB id: 1PCR [18]) and two PufX proteins [83] were finally placed within the LH1 dimer based on EM data [57] (see [64] for further details of the modeling effort).

The atomic structural model for the RC–LH1–PufX dimer thus constructed in [64] was fitted into the 25 Å resolution 3D EM density map reported in [59] as follows. Rigid-body docking was first performed to place the dimer model inside the EM density using the Situs package [84]. A 320 Å × 170 Å lipid patch composed of 50% POPE and 50% POPG was then positioned around the dimer. The lipid–protein system was solvated in a 350 Å × 200 Å × 140 Å water box. Ions were added at 0.3 M to mimic the physiological environment. The entire solvated and membrane-embedded RC–LH1–PufX dimer system consists of 890,307 atoms. The simulation setup is shown in Fig. 1.

The MDFF simulation was performed using NAMD [85–88]. Parameters for the MD potential (U_{MD}) were taken from the CHARMM27 force field [89] with the CMAP correction [90], water being described by the TIP3P model [91]. Long-range electrostatic forces were evaluated using the particle-mesh Ewald (PME) summation method with a grid size of < 1Å. An integration time step of 1 fs was used with a multiple time-stepping algorithm [92]. Bonded terms were evaluated every time step, with short-range non-bonded terms evaluated every fourth time step. Constant temperature ($T = 300$ K) was maintained using Langevin dynamics, with a damping coefficient of 1 ps^{-1}. A constant pressure of 1 atm was enforced using a Nosé–Hoover Langevin piston with a decay period of 100 fs and time constant of 50 fs.