Biochimica et Biophysica Acta 1807 (2011) 157–164

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Structural model and spectroscopic characteristics of the FMO antenna protein from the aerobic chlorophototroph, *Candidatus* Chloracidobacterium thermophilum

Jianzhong Wen [a,b], Yusuke Tsukatani [c], Weidong Cui [b], Hao Zhang [b], Michael L. Gross [b], Donald A. Bryant [c], Robert E. Blankenship [a,b,*]

[a] Department of Biology, Washington University in St. Louis, St. Louis MO 63130, USA
[b] Department of Chemistry, Washington University in St. Louis, St. Louis MO 63130, USA
[c] Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA

ARTICLE INFO

Article history:
Received 9 August 2010
Received in revised form 15 September 2010
Accepted 17 September 2010
Available online 25 September 2010

Keywords:
Acidobacteria
Type-1 reaction center
FMO protein
Baseplate
Native-electrospray mass spectrometry
Homology modeling

ABSTRACT

The Fenna–Matthews–Olson protein (FMO) binds seven or eight bacteriochlorophyll *a* (BChl *a*) molecules and is an important model antenna system for understanding pigment-protein interactions and mechanistic aspects of photosynthetic light harvesting. FMO proteins of green sulfur bacteria (*Chlorobiales*) have been extensively studied using a wide range of spectroscopic and theoretical approaches because of their stability, the spectral resolution of their pigments, their water-soluble nature, and the availability of high-resolution structural data. We obtained new structural and spectroscopic insights by studying the FMO protein from the recently discovered, aerobic phototrophic acidobacterium, *Candidatus* Chloracidobacterium thermophilum. Native C. thermophilum FMO is a trimer according to both analytical gel filtration and native-electrospray mass spectrometry. Furthermore, the mass of intact FMO trimer is consistent with the presence of 21–24 BChl *a* in each. Homology modeling of the C. thermophilum FMO was performed by using the structure of the FMO protein from *Chlorobaculum tepidum* as a template. C. thermophilum FMO differs from *C. tepidum* FMO in two distinct regions: the baseplate, CsmA-binding region and a region that is proposed to bind the reaction center subunit, PscA. C. thermophilum FMO has two fluorescence emission peaks at room temperature but only one at 77 K. Temperature-dependent fluorescence spectroscopy showed that the two room-temperature emission peaks result from two excited-state BChl *a* populations that have identical fluorescence lifetimes. Modeling of the data suggests that the two populations contain 1–2 BChl and 5–6 BChl *a* molecules and that thermal equilibrium effects modulate the relative population of the two emitting states.

1. Introduction

Photosynthesis is a central biological process that produces all the food and much of the energy used by human beings. Intense attention has been focused on using photosynthetic organisms or mechanisms adapted from photosynthesis as sources to produce cheap, clean and renewable energy [1–4]. The photosynthetic process starts with photon capture by a group of pigment-binding protein complexes called light-harvesting complexes. In these complexes, energy from absorbed photons is converted to excitation energy, which is subsequently transferred to reaction centers (RC), where it is transduced into chemical potential energy through photochemically induced, redox reactions that ultimately lead to the synthesis of ATP and in many cases to the reduction of inorganic carbon to organic compounds [5].

An enormous amount of work has been done to understand the structural basis of light harvesting and the efficiency of the energy-transfer process [6–8]. Several bacterial light-harvesting (LH) complexes, such as the LH1 [9,10] and LH2 [11,12] complexes from purple bacteria and the Fenna-Matthews-Olson protein (FMO) from green sulfur bacteria [13,14], have served as model systems for structural, spectroscopic and theoretical studies. These investigations have deepened our understanding of pigment-protein interactions and how these complexes achieve photon capture and high energy-transfer efficiencies. On the other hand, the discovery of new photosynthetic species and new light-harvesting complexes has greatly expanded our vision of the evolution of photosynthesis and also provides the means to explore new systems [15–18].

The recently discovered, thermophilic bacterium, *Candidatus* Chloracidobacterium (C.) thermophilum, belongs to the phylum *Acidobacteria*, and is the only species found in the phylum so far that is a phototroph [16]. Surprisingly, the photosystem of the aerobic

* Corresponding author. Departments of Biology and Chemistry, Campus Box 1137, Washington University in St. Louis, St. Louis, MO 63130, USA. Tel.: +1 314 935 7971; fax: +1 314 935 4432.
E-mail address: blankenship@wustl.edu (R.E. Blankenship).

0005-2728/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2010.09.008

C. thermophilum closely resembles that of the green sulfur bacteria (GSB), which are strict anaerobes. The energy harvested by the large peripheral antenna complex called the chlorosome [19,20] is transferred through the FMO protein to a type-I reaction center in the C. thermophilum cells, in a manner similar to that in GSB. The cells of GSB, however, are susceptible to light damage under aerobic conditions [19], because reactive oxygen species are readily produced from reduced ferredoxin generated from the type-I RCs. The mechanism that enables the C. thermophilum cells to carry out phototrophy under aerobic conditions is not yet known.

The initial characterization of C. thermophilum FMO by Tsukatani et al. [21] demonstrated its apparent difference from the more commonly studied GSB FMO proteins. The C. thermophilum FMO is distantly related to the GSB FMOs, and is about 60% similar in sequence to the FMO proteins of GSB, which are approximately 80% similar to one another. As shown by its weaker CD signal, the excitonic coupling of the bacteriochlorophyll (BChl) *a* molecules in the C. thermophilum FMO is somewhat weaker than that in the GSB FMOs [21]. Another interesting phenomenon is that the C. thermophilum FMO exhibits two fluorescence peaks at room temperature (RT), whereas GSB FMO proteins always have a single fluorescence emission peak.

In the research reported here, we further investigated the C. thermophilum FMO protein both structurally and spectroscopically. Firstly, we determined the overall size of the C. thermophilum FMO by analytical gel filtration and native electrospray mass spectrometry, which allows the molecular weight of the complex to be measured, thus defining the stoichiometry of the interacting components [22–25]. Secondly, we applied homology modeling [26] to generate a structural model of the C. thermophilum FMO using the known crystal structure of FMO from *Chlorobaculum tepidum* (*C. tepidum*) as a template. The overall similarity of the two sequences is 57–59% (sequence identity of ~40%) [21]. In general, with this level of similarity, homology modeling is quite feasible and can provide structural information in the absence of an X-ray structure. The constructed model allowed us to identify two regions that differ significantly from the *C. tepidum* FMO crystal structure. One region likely interacts with CsmA in the baseplate, and the other region may interact with the RC. Finally, we measured the temperature-dependent, steady-state fluorescence emission spectra and the excited state lifetimes to understand better the origin of the two fluorescence peaks reported earlier [21]. We found that the two emission peaks are associated with two thermally equilibrated populations of excited states at RT.

2. Materials and methods

2.1. Sample preparation and analytical gel filtration

C. thermophilum cells were grown, and FMO protein was purified by the procedures described previously [21]. The purified protein was loaded onto an analytical gel filtration column (ZORBAX GF-250, 4.6×250 mm, 150 Å pore diameter, 4 μm particle size, Agilent) attached to an Agilent series 1100C high-performance liquid chromatography (HPLC) system. The equilibration and the elution buffer was 0.1 M sodium phosphate buffer (pH = 7.0) with 0.15 M sodium chloride at a flow rate of 0.5 mL/min. FMO sample (10 μL) with optical density at 806 nm of approximately 10 in a 1 cm path cuvette ($OD_{806\ nm}$ ~ 10 cm^{-1}) was injected by using an auto sampler. Lactate dehydrogenase, catalase, conalbumin and carbonic anhydrase were purchased from Sigma (St. Louis, MO USA) and used as references to calibrate the column. They were resuspended in phosphate buffer to 10 μM, and 10 μL of each was loaded onto the column. The photodiode-array detector was set to detect 810, 600, 370, 280 and 214 nm.

2.2. Mass spectrometry analysis

MS measurements of the native and denatured FMO protein were carried out on a 12 Tesla Fourier transform ion-cyclotron resonance (FTICR) mass spectrometer equipped with a nano-electrospray source (SolariX, Bruker DaltoniK GmbH, Bremen, Germany) coupled either to a PHD ULTRA™ syringe pump (Harvard Apparatus, Holliston, MA), or to a nano-ACQUITY UltraPerformance LC (Waters Corp., Milford, MA).

For the native electrospray, the C. thermophilum FMO complex was exchanged into 0.75 M ammonium acetate buffer (pH = 7.5) and concentrated to ~8 μM. Sample infusion was performed by nano-electrospray (nano-ESI), using a custom-pulled silica capillary needle at a voltage of 850–1500 V. The needle was pulled by the P-2000 Laser Puller (Sutter Instrument CO., Novato, CA) using fused silica capillary tubing with ID 150 μm (Polymicro Technologies LLC, Phoenix, AZ). The flow rates for the mass spectral measurements were between 20 nL/min and 0.1 μL/min. Optimization of the ion transfer parameters and calibration of the instrument to $m/z = 8000$ were carried out by direct infusion of 5 mM cesium perfluoroheptanoate. To achieve better native electrospray signals, the capillary voltage was lowered to 850–1200 V once the spray was initiated at 1500 V and kept stable. The collision energy in the collision cell was increased to 20–40 eV to observe good native electrospray signals. For acquisition of some spectra, the in-source collision-induced dissociation was turned on, and collision voltages up to 25 V were used, which helped to desolvate the complex without dissociating it.

To measure the mass of the denatured C. thermophilum FMO polypeptide, a protein aliquot (10 μl of a ~0.5 μM solution) was loaded onto an Opti-Guard trap column (Optimize Technologies, INC., Oregon City, OR) and washed by 0.1% formic acid (FA) in water before it was eluted by using 80% acetonitrile with 0.1% FA. The ESI conditions were: positive-ion mode; capillary voltage, 4000 V; dry gas, 5 L/min; and dry gas temperature, 150 °C.

Charge deconvolution of the ESI mass spectrum of the denatured C. thermophilum FMO was determined by MagTran algorithm [27]. The charge deconvolution of the native electrospray spectrum was manually calculated. For the simulation of the native electrospray spectrum, a Gaussian function with a bandwidth of 60 m/z at each charge state was applied by using Origin (OriginLab Corporation, Northampton, MA).

2.3. Homology modeling

Homology modeling of the C. thermophilum and *Chloroherpeton thalassium* FMO proteins was carried out using the web-based program SwissModel (http://swissmodel.expasy.org/) under automated mode. The atomic structure of FMO from *C. tepidum* (TFMO, PDB ID: 3BSD) was automatically selected as the template. The generated PDB file of the C. thermophilum FMO structure was viewed and aligned with the crystal structure of TFMO (PDB ID: 3ENI) by Pymol (Version 1.2r3pre, Schrödinger, LLC).

The alignment of CsmA sequences was performed using the ClustalW2 program on the website http://www.ebi.ac.uk/Tools/clustalw2/index.html. Phylogenetic analyses were carried out using the MEGA4 [28] program. Construction of the phylogenetic tree was performed by the neighbor-joining method applying the p-distance parameter.

2.4. Temperature-dependent fluorescence emission and fluorescence lifetime measurements

The fluorescence emission was measured using a setup previously described by us [29]; the excitation wavelength was 370 nm. The sample in 70% (v/v) glycerol with $OD_{806\ nm} = 0.1\ cm^{-1}$ was placed in a cryostat (OptistatDN, Oxford Instrument, USA), and the temperature of the cryostat was controlled by the Intelligent Temperature