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The structure of the FMO protein from *Chlorobium tepidum* at 2.2 Å resolution

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Abstract

The bacteriochlorophyll protein, or FMO protein, from *Chlorobium tepidum*, which serves as a light-harvesting complex and directs light energy from the chlorosomes attached to the cell membrane to the reaction center has been crystallized in a new space group. The crystals belong to the cubic space group $P4_332$ and the structure has been refined to a resolution 2.2 Å with a R factor of 19.7%. The electron density maps show that the structure is composed of two β sheets that surround seven bacteriochlorophylls as previously reported (Li et al. (1997) J Mol Biol 271: 456–471). The availability of the new data allows a more accurate refinement of the pigment–protein complex including identification of bound solvent molecules. Several structural differences probably contribute to the observed spectroscopic differences between the FMO proteins from *Cb. tepidum* and *Prosthecochloris aestuarii,* including differences in the planarity of corresponding tetrapyrroles. A citrate molecule is found on the surface of each protein subunit of the trimer from *Cb. tepidum*. However, the citrate molecule is over 15 Å from any bacteriochlorophyll. The presence of the citrate probably does not contribute to the function of the protein although it does contribute to the crystallization as it interacts with a crystallographically related trimer. Among the 236 water molecules found in the protein are four that appear to play a special role in the properties of bacteriochlorophyll 2, as this tetrapyrrole is coordinated by one of these water molecules and the waters form a hydrogen-bonded network that leads to the surface of the protein.

Introduction

Green anoxygenic photosynthetic bacteria are composed of two major classes, Chlorobiaceae and Chloroflexaceae. Of all of the photosynthetic bacteria, only these two classes possess the unique chlorosome antenna. Chlorosomes are complexes approximately 100–200 nm in length attached to the cytoplasmic side of the cell membrane. Each chlorosome contains up to 200,000 bacteriochlorophylls that are organized to capture light energy and direct it to the reaction center where energy conversion occurs (Blankenship et al. 1995; Olson 1998; Blankenship and Matsuura 2003). In green non-sulfur bacteria, a bacteriochlorophyll *a* containing protein known as the Fenna–Matthews–Olson (FMO) protein is located between the chlorosome and reaction center. The FMO protein was first discovered in *Prosthecochloris (Pc.) aestuarii* (Olson and Romano 1962; Olson 1994). The FMO protein from *Pc. aestuarii* was crystallized (Olson 1978) and its structure was determined initially at a resolution of 2.8 Å (Fenna and Matthews 1975; Matthews et al. 1979) and subsequently at a resolution limit of 1.9 Å (Tronrud et al. 1993).

The FMO protein from *Chlorobium (Cb.) tepidum* was later crystallized and the structure was determined to a resolution limit of 2.2 Å (Li et al. 1997). Overall, the two structures are very similar with both

structures consisting of trimers of identical subunits. Each of the subunits is organized as a 'taco shell' of two β sheets surrounding seven bacteriochlorophylls. The distances between the bacteriochlorophylls were found to range from 4 to 11 Å within each subunit and the distances between bacteriochlorophylls from different subunits are over 20 Å. The similarity of the structures agrees well with the high sequence identity of 78% (Daurat-Larroque et al. 1986; Dracheva et al. 1992). The orientation of the trimer in the membrane was suggested by the presence of a hydrophobic region on the surface that suggests the complex as being partially embedded in the membrane. This overall orientation was confirmed by linear dichroism measurements although which face of the FMO protein faces the chlorosome is still not unambiguously established (Melkozernov et al. 1998). Electron microscopic studies suggest that two FMO proteins are associated with each reaction center (Remigy et al. 2002).

Despite the overall structural homology, the FMO proteins from *Cb. tepidum* and *Pc. aestuarii* have distinctive spectral features (Francke and Amesz 1997; Vulto et al. 1998a, b). Spectroscopic measurements suggest differences in the interactions among the bacteriochlorophylls in the two complexes (Olsen et al. 1978; Johnson and Small 1991; Wendling et al. 2002). Computer simulations have suggested that these spectral differences arise from small differences in the energies of the bacteriochlorophylls (Louwe et al. 1997a, b; Iseri and Gulen 1999) that arise from small differences in the protein environments and planarity of the bacteriochlorophylls. Thus, models of these spectroscopic features are strongly dependent upon the interactions among the seven bacteriochlorophylls of each subunit and hence the quality of the three dimensional structures. The quality of the original structure of the FMO protein from *Cb. tepidum* was limited by a slight disorder in the crystals. To improve on the accuracy of the structure, data has been measured in a new space group that does not possess the disorder. The structure has been refined using the new data and the structural differences between the FMO protein from *Cb. tepidum* in the new crystal form and the structure of *Pc. aestuarii* are discussed. The implications of new features of the model for the properties of the protein are presented.

Table 1. Summary of diffraction and structural data

Space group	$P4_332$
Cell parameter $a= b= c$ (Å)	169.1
Resolution range (Å)	20.0 – 2.2
Total reflections	247 830
Unique reflections	41 641
R_{merge} (%)[a]	12.1 (57.3)
Completeness (%)[a]	91.5 (77.4)
R_{cryst}, R_{free}(%)	19.7, 23.9
R.m.s. bond deviations (Å)	0.008
R.m.s. angle deviations (°)	1.6
Ramachradran plot	
Most favorable (%)	90.1
Additionally allowed (%)	9.9
Not allowed (%)	0.0
Average B-factor (Å^2)	
Main chain atoms	26.7
Side chain atoms	29.3

[a]Overall data with last 0.1 Å shell in parentheses.

Materials and methods

The protein was purified as previously described (Li et al. 1997). Crystals were obtained using the vapor diffusion technique. The protein solution consisted of 13 mg/ml protein, 10 mm Tris pH 8.6% (w/v) polyethylene glycol 600, 50 mM sodium citrate pH 5.6, and 10% (v/v) 2-proponal. The reservoir consisted of 12% polyethylene glycol 600, 100 mM sodium citrate, and 20% 2-proponal. The crystals grew to a maximal size of 0.8 mM in 2 weeks. This crystallization follows the same conditions as previously described (Li et al. 1997) except polyethylene glycol 600 was used instead polyethylene glycol 4000. This change in the crystallization conditions leads to crystals of a similar size and shape but with the space group $P4_332$ with cell constants of 169.1 Å compared to the previously obtained crystals in the space group $P4_132$ with cell constants of 169.5 Å. Data were collected using graphite-filtered copper radiation using a rotating anode and a RAXIS II (Molecular Structure Corporation) area detector. The data set was measured from one crystal yielding 247,830 total observations and 41,641 unique reflections with a R_{sym} of 12.1% that is 91.5% complete to a resolution limit of 2.2 Å (Table 1). The data were integrated and scaled using the HKL programs (Otwinowski and Minor 1997). The quality of the model was examined using PROCHECK (Laskowski et al. 1993). The coordinates for the two FMO structures was superimposed using the program