Crystallization and preliminary X-ray diffraction analysis of the peripheral light-harvesting complex LH2 from *Marichromatium purpuratum*Laura J. Cranston,^a
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Accepted 24 April 2014

LH2 from the purple photosynthetic bacterium *Marichromatium* (formerly known as *Chromatium*) *purpuratum* is an integral membrane pigment–protein complex that is involved in harvesting light energy and transferring it to the LH1–RC ‘core’ complex. The purified LH2 complex was crystallized using the sitting-drop vapour-diffusion method at 294 K. The crystals diffracted to a resolution of 6 Å using synchrotron radiation and belonged to the tetragonal space group *I*4, with unit-cell parameters $a = b = 109.36$, $c = 80.45$ Å. The data appeared to be twinned, producing apparent diffraction symmetry *I*422. The tetragonal symmetry of the unit cell and diffraction for the crystals of the LH2 complex from this species reveal that this complex is an octamer.

1. Introduction

The peripheral light-harvesting complex LH2 is a major component of the photosynthetic unit in purple bacteria (Gabrielsen *et al.*, 2009). Multiple LH2 complexes located in the intracytoplasmic membranes harvest light energy and pass it on to the reaction centre RC *via* light-harvesting complex LH1 (Gabrielsen *et al.*, 2009). The LH2 complex is a ring-like oligomer of a basic unit which consists of a pair of small (~6 kDa) hydrophobic apoproteins (named α and β). These proteins noncovalently bind two major light-absorbing pigments: bacteriochlorophylls (Bchl *a*) and carotenoids (Cogdell *et al.*, 1999). The absorption spectra of LH2 complexes depend on their pigment composition and on how these pigments interact with each other and with the apoproteins in which they are embedded. These absorption spectra can differ significantly between the various purple bacterial species.

To date, there are only two high-resolution structures of LH2 complexes available, and they show different oligomeric symmetry. The structure of LH2 from *Rhodospseudomonas acidophila* strain 10050, known also as the B800–850 complex owing to its absorption maxima at 800 and 850 nm, has been determined to 2.0 Å resolution (Papiz *et al.*, 2003). It has a nonameric ring symmetry with nine identical α/β -heterodimers each binding three BChl *a* molecules and one carotenoid molecule. The LH2 structure from *Phaeospirillum* (previously *Rhodospirillum*) *molischianum* (resolved to 2.4 Å resolution) has an overall similar composition except for its octameric symmetry (Koepke *et al.*, 1996). The LH2 complex from the low-light-adapted *Rps. acidophila* strain 7050 (known as the B800–820 complex owing to its absorption maxima at 800 and 820 nm; structure determined to a resolution of 3 Å; McLuskey *et al.*, 2001) forms analogous nonameric rings very similar to the LH2 complex from strain 10050. The observed spectroscopic differences between the LH2 complexes from these two *Rps. acidophila* strains can be attributed to differences in the apoprotein sequences and changes in hydrogen-bonding patterns to the BChl *a* molecules (McLuskey *et al.*, 2001; Gabrielsen *et al.*, 2009).

The absorption spectrum of the LH2 complex from the purple sulfur photosynthetic bacterium *Marichromatium* (formerly known as *Chromatium*) *purpuratum* shows near-infra-red (NIR) maxima at 800 and 830 nm (Cogdell *et al.*, 1990). This spectrum is shown and compared in Fig. 1 with the spectra of the LH2 complexes from

Rps. acidophila strains 10050 and 7050 (Gardiner *et al.*, 1993). The absorption spectrum for the *Phs. molischianum* LH2 complex (not shown) is essentially the same as that for the complex from *Rps. acidophila* strain 10050 (Germeroth *et al.*, 1993). The differences between these LH2 spectra in their central region (450–600 nm) are owing to the presence of different carotenoids in each complex: okenone, rhodopin glucoside and rhodopinal glucoside for *M. purpuratum*, *Rps. acidophila* strain 10050 and *Rps. acidophila* strain 7050, respectively (Cogdell *et al.*, 1990; Gardiner *et al.*, 1993). The differences in the NIR region (750–900 nm) are owing to differences in interactions between the BChl *a* molecules and the apoproteins (Gabrielsen *et al.*, 2009). In order to try to discover the structural features of the LH2 from *M. purpuratum* that are responsible for the differences in the NIR absorption spectra observed in Fig. 1, the LH2 from *M. purpuratum* has been purified and crystallized in an attempt to determine its three-dimensional structure. The symmetry of the diffraction data for the crystals obtained, together with their unit-cell dimensions, has allowed the symmetry of the LH2 ring for this species to be determined with high confidence.

2. Experimental

2.1. Protein purification

Cells of *M. purpuratum* strain BN5500 (also designated DSM1591 or 984) were grown anaerobically in the light, harvested by centrifugation and suspended in 20 mM Tris–HCl pH 8.0. Then, upon the addition of DNase and MgCl₂, the cells were broken by passage through a French press. The photosynthetic membranes were collected from the broken-cell mixture by centrifugation and resuspended in the Tris–HCl solution to an optical density (OD) of 50 at 830 nm. The membranes were then solubilized by the addition of 1% (v/v) of the detergent *N,N*-dimethyldodecylamine-*N*-oxide (LDAO). These solubilized membranes were subjected to sucrose-

density centrifugation with a step gradient (as described previously by Brotosudarmo *et al.*, 2011) in order to fractionate the sample into LH2 and RC–LH1 complexes. The LH2 fraction was then further purified by ion-exchange chromatography using diethylaminoethyl cellulose (DE52, Whatman). The bound protein was washed with 20 mM Tris–HCl pH 9.0 containing 0.15% dodecyl maltoside (DM) to exchange the LDAO detergent and then eluted by adding increasing concentrations of NaCl also containing 0.15% DM. Additional purification was achieved by gel-filtration chromatography using a Superdex S-200 column (XK 16/100, GE Healthcare) which had been equilibrated overnight with 20 mM Tris–HCl pH 9.0 plus 0.15% DM. Fractions that had an absorbance ratio A_{830}/A_{270} of >2.2 were pooled together and concentrated to an OD_{830 nm} of 100 using Vivaspinn2 Centricons with a 50 kDa molecular-weight cutoff. The integrity of the purified LH2 complex was checked spectrophotometrically at the beginning of the purification process, after every purification step and after the crystals had been redissolved by measuring the absorption spectrum from 250 to 950 nm with a spectrophotometer (Shimadzu UV-1700). No denaturation was observed.

2.2. Crystallization

Crystallization was performed by the vapour-diffusion method in 96-well plates using the MemGold and MemStart/MemSys crystallization screens (Molecular Dimensions) and a Cartesian HoneyBee 8+1 nanovolume robot. Sitting drops were prepared by mixing 0.5 µl of the purified protein (OD_{830 nm} of 100 and 50) in 20 mM Tris–HCl pH 9.0, 0.15% DM with 0.5 µl of the reservoir solution and were equilibrated against 50 µl reservoir solution at 294 K, stored and periodically scanned in a Rhombix Imager (Thermo Fisher Scientific). Deep purple, square-by-pyramidal crystals appeared after six weeks from condition 2.37 of the MemGold screen, which consisted of 33% (v/v) PEG 300, 0.1 M sodium phosphate pH 7.0, 0.1 M sodium chloride, and grew to their final size within the next two weeks.

2.3. X-ray data collection and processing

Crystals of approximately 200 × 200 × 100 µm in size (Fig. 2) were scooped up from a droplet in LithoLoops (Molecular Dimensions) and directly flash-cooled in a nitrogen-gas stream at 100 K. X-ray data were collected from a single crystal at a temperature of 100 K on the I02 beamline at the Diamond Light Source (DLS) equipped with an ADSC Quantum 315r detector. 180 images were collected at a wavelength of 0.9795 Å using 1.0° rotations (Fig. 3). At the DLS the data integrated by *XDS* (Kabsch, 2010) were judged by *POINTLESS*

Figure 1
Absorption spectra of LH2 complexes from *Rps. acidophila* strain 10050 (orange, bottom), *Rps. acidophila* strain 7050 (blue, middle) and *M. purpuratum* (purple, top); these spectra have been offset along the vertical axis for clarity.

Figure 2
Crystal of the LH2 complex from *M. purpuratum* used for data collection.

(Evans, 2006) and *XSCALE* (Kabsch, 2010) to belong to space group *I422*, and were subsequently reduced to *I422* by the automated data-reduction pipeline *xia2* (Winter, 2010). However, as explained in detail below in §3, this *I422* data set was judged to be an artifact of merohedral twinning of the *I4* crystal, which produced apparent diffraction of higher symmetry (Koch, 2004). In order to obtain lower symmetry data, reprocessing of the original images was performed in space groups *P1*, *I4* and *I422* using *d*TREK* (Pflugrath, 1999). The processing of the data in *P1* was for the purpose of detection of the unbiased symmetry of the LH2 complex using the self-rotation function and for molecular-replacement trials. Statistics for the *d*TREK* processing in space group *I422* were similar to those from earlier *xia2* processing (not shown). *phenix.xtriage* (Zwart, 2005) was used to verify the twinning. A summary of the data collection from a single crystal and the *d*TREK* processing statistics of this data set merged in the three space groups is given in Table 1.

2.4. Self-rotation function and molecular replacement

The self-rotation functions for the data merged in three reported space groups were calculated using *MOLREP* (Vagin & Teplyakov, 2010). Molecular replacement (MR) was performed using *Phaser* (McCoy *et al.*, 2007). Additional calculations were performed using tools from the *CCP4* suite (Winn *et al.*, 2011) and the *PHENIX* suite (Adams *et al.*, 2010). Refinement in 'jelly-body' mode was performed using *REFMAC5* (Murshudov *et al.*, 2011).

3. Results and discussion

A comparison of the sequences of the α/β -apoproteins of the LH2 complex from *M. purpuratum* (*purp*-LH2) with those of the LH2 complexes from *Rps. acidophila* (*ac*-LH2) and *Phs. molischianum* (*mol*-LH2) shows a high degree of homology (Henry & Cogdell, 2013). It is therefore to be expected that the basic structure of the

Table 1

Data-collection and processing statistics for the *d*TREK* processing of data from a single crystal of *M. purpuratum* LH2.

Three columns show statistics for data reduction in three different space groups. Values in parentheses are for the outermost resolution shell.

Space group	<i>P1</i>	<i>I4</i>	<i>I422</i>
Unit-cell parameters			
<i>a</i> (Å)	80.36		109.36
<i>b</i> (Å)	86.93	109.36	109.36
<i>c</i> (Å)	87.17	80.45	80.45
α (°)	77.85	90	90
β (°)	62.58	90	90
γ (°)	62.82	90	90
Resolution range (Å)	27.95–6.67 (6.91–6.67)	25.78–6.63 (6.87–6.63)	25.78–6.61 (6.84–6.61)
Observed reflections	5996 (614)	6165 (635)	6211 (667)
Unique reflections	3183 (322)	894 (87)	518 (53)
Average multiplicity	1.9 (1.9)	6.9 (7.4)	12.0 (12.7)
Completeness (%)	94.6 (99.4)	99.2 (100)	99.4 (100)
Mean $I/\sigma(I)$	7.3 (1.5)	9.5 (1.6)	10.4 (1.5)
$R_{\text{merge}}^{\dagger}$ (%)	3.5 (36.2)	5.3 (54.1)	5.9 (59.7)
$R_{\text{meas}}^{\ddagger}$ (%)	5.0 (51.2)	5.8 (58.3)	6.2 (62.3)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the mean intensity for the unique reflection *hkl*. $\ddagger R_{\text{meas}} = \sum_{hkl} [N(hkl)/[N(hkl) - 1]]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $N(hkl)$ is a total number of observations for the reflection *hkl* used in the statistics.

purp-LH2 complex will have a similar ring-like oligomeric structure to the *ac*-LH2 and *mol*-LH2 complexes (Papiz *et al.*, 2003; Koepke *et al.*, 1996); however, the oligomeric symmetry of this ring was unknown prior to the present study. Both the *ac*-LH2 and the *mol*-LH2 complexes have a hollow cylindrical topology with approximate cylinder diameter and height (including the C-termini) dimensions of 80 and 70 Å, respectively, for nonameric *ac*-LH2 and 75 and 65 Å, respectively, for octameric *mol*-LH2. We therefore tested these two structures as search models in MR attempts to phase the diffraction data for *purp*-LH2.

An additional feature of the crystal structures of the *ac*-LH2 and *mol*-LH2 complexes is that the LH2 rings form dimeric pairs within the crystal lattice. These tail-to-tail dimers are formed by an intercalation of the C-termini of α -polypeptides so their overall lengths are shorter than twice the lengths of the individual LH2 complexes. The *ac*-LH2 dimer is about 112 Å in length, while the *mol*-LH2 dimer is about 120 Å in length, with the difference coming mainly from the difference in the extent of intercalation of the C-termini and the differences in the lengths of the respective α/β -polypeptide sequences (53/41 amino acids for *ac*-LH2 and 56/46 for *mol*-LH2). The dimers of nonameric *ac*-LH2 are located around the threefold axes in the *H32* space group with the two monomers related by the crystallographic twofold axis, while the dimers of octameric *mol*-LH2 with two independent monomers (related by a pseudo-twofold axis) are located around the fourfold axes in the *P4₂2* space group. If the *purp*-LH2 complexes form dimers in the crystal lattice as well, their length would be expected to be at least 100 Å but would probably be even longer than 120 Å because its α/β -polypeptides are composed of 70–71/44–49 amino acids, respectively (*M. purpuratum* gene; GenBank CP007031.1; expressed α -polypeptides partially truncated at the C-termini).

The data collected for the crystal of *purp*-LH2 were originally indexed in the *I422* space group with the unit-cell dimensions as shown in the last column of Table 1. In a unit cell of such small volume, 962 150 Å³, and with a symmetry with 16 asymmetric units, the *purp*-LH2 complex would have to be positioned in a special position around the fourfold axis, and therefore should have an octameric oligomeric form. Moreover, the volume of the asymmetric unit of this cell, ~60 000 Å³, is too small to accommodate a quarter of

Figure 3

An example of an X-ray diffraction image for an LH2 crystal from *M. purpuratum*; reflections were observed up to 6 Å resolution.

the octameric LH2 ring, especially when assuming the same $\sim 70\%$ solvent content as observed in the *ac*-LH2 and *mol*-LH2 structures. The fraction of the unit cell of *mol*-LH2 per quarter of the octameric LH2 ring is $\sim 110\,000\text{ \AA}^3$, almost twice the volume of the asymmetric unit in the apparent $I422$ cell of *purp*-LH2. Additionally, the LH2 ring is different at each end and therefore cannot have 422 point-group symmetry with the twofold axis perpendicular to the long axis of the ring. A dimer of two octameric rings could have such symmetry, but its likely length in excess of $100\text{ \AA}$ cannot be accommodated along the

c unit-cell axis of the *purp*-LH2 crystal ($80.45\text{ \AA}$). These were the reasons why the data were reprocessed in the lower symmetry $I4$ and also in $P1$ symmetry to check the symmetry of data without any bias and for use in MR trials. The apparent higher symmetry $I422$ of the diffraction data is possibly owing to twinning by merohedry of the *purp*-LH2 crystal, of which the true symmetry is $I4$ (Koch, 2004). Indeed, according to *phenix.xtriage* (Zwart, 2005) the $I4$ -merged data show ‘intensity statistics which are significantly different than expected from good to reasonable, untwinned data’ with an L -test

Figure 4 Stereographic projections of the self-rotation function for (a) $P1$ -merged data, $\chi = 180^\circ$; (b) $P1$ -merged data, $\chi = 90^\circ$; (c) $I4$ -merged data, $\chi = 180^\circ$; (d) $I4$ -merged data, $\chi = 90^\circ$.

multivariate Z -score of 8.9 (3.5 is expected for 'good to reasonable data'), possibly owing to merohedral twinning with the twin law $-k, -h, -l$ and with the twin fraction estimated as between 0.32 (Britton analysis) and 0.476 (maximum likelihood). The intensity statistics for the $I422$ -merged data showed an L -test multivariate Z -score of 14.8 and the $I422$ -data were judged to be 'overmerged pseudo-symmetric or twinned'.

Fig. 4 shows stereographic projections of the self-rotation function calculated for the $P1$ -merged data in Figs. 4(a) and 4(b), and $I4$ -merged data in Figs. 4(c) and 4(d). Fig. 4(b) reveals the presence of the fourfold axis approximately along the unit-cell axis a , which is 80.36 Å in length. The c axis in the $I4$ cell, along which the fourfold axis extends, is about the same length at 80.45 Å. Fig. 4(a) shows an extended 'belt' perpendicular to the fourfold axis with the stronger peaks within that 'belt' indicating the directions of twofold axes 45° apart. These features represent both the tetragonal symmetry of the data and the orientation of the octameric LH2 ring in the crystal lattice. The self-rotation function calculated for the $I4$ -merged data (Figs. 4c and 4d) confirms the unique orientation of an octameric LH2 ring within the $I4$ crystal lattice with the ring axis oriented along the unit-cell axis c . A continuous 'belt' of the NCS twofold symmetry visible in Fig. 4(a) (horizontal feature in the middle) and Fig. 4(c) (feature along/at the projection perimeter) represents orientations of the approximate twofold axes perpendicular to the long axis of the LH2 'cylinder', roughly symmetrical at this limited resolution. The self-rotation function calculated for the data merged in $I422$ is identical to that shown for data merged in $I4$.

MR for the $P1$ -merged data was performed using the two available high-resolution full-ring models of *ac*-LH2 and *mol*-LH2, as well as two full-ring models of octameric and dodecameric LH2 which were generated using α/β -heterodimers of *ac*-LH2. The MR solutions for all of these models were obtained and found to be oriented with their

ring axis approximately along the unit-cell axis a , *i.e.* in agreement with the self-rotation function. The values of the figure of merit LLG calculated by *Phaser* (McCoy *et al.*, 2007) are 71, 59, 63 and 52 for *ac*-LH2, *mol*-LH2, 'octameric *ac*-LH2' and 'dodecameric *ac*-LH2', respectively, assuming a solvent content of 70%. A close examination of the crystal packing for these solutions shows however that the 'dodecameric *ac*-LH2' model is too large to be accommodated without clashes, considering that the real LH2 complex is surrounded by the detergent micelle, which is not present in the MR model. The packing of the other three models shows no clashes and leaves a space for detergent micelles.

MR for the $I4$ -merged data was also attempted using a quarter of the octameric models mentioned above, *i.e.* of the *mol*-LH2 model and of the 'octameric *ac*-LH2'. These 'quarter of a ring' models contain only two α/β -heterodimers with their associated Bchl a and carotenoid molecules. The only reasonable solutions, which form complete '*purp*-LH2' rings about the fourfold axes, were obtained for the quarter of the 'octameric *ac*-LH2'. In this case *Phaser* (McCoy *et al.*, 2007) gave exactly two solutions (with LLG 30.9 and 29.6, assuming a solvent content of 70%), which are related by a pseudo-twofold axis oriented along the crystal axis a and are most likely to be a consequence of the crystal twinning and apparent $I422$ data symmetry. Effectively, the '*purp*-LH2' rings for these two solutions are identical but oriented in two opposite directions along the fourfold axis. Crystal packing for the first of these MR solutions viewed along the fourfold axis is presented in Fig. 5.

Restraint refinement of this MR solution with *REFMAC5* using the 'jelly-body' mode (Murshudov *et al.*, 2011) produced only a marginal improvement of the R factor (from 63.4 to 56.2% after 243 cycles). Assuming that the data twinning does affect this refinement, a test refinement has been performed with the two overlapping components related by a twofold NCS present in the crystal lattice at the same time. For this, both MR $I4$ solutions were used in the refined 'model' with occupancies of 60 and 40%, respectively. This refinement converged after 90 cycles with the R factor decreasing from 63.2 to 50.9% (refinement with twinning fractions of 50/50% reduced R to 51.2%). Rebuilding of the two-component 'model' was not attempted because of this low resolution.

Crystals of the *purp*-LH2 complex diffracting to higher resolution are needed to resolve the details of its structure. A search for appropriate amphiphiles and/or different detergents is continuing to try to improve the crystals. The limited resolution data obtained to date are already sufficient to allow us to determine that the oligomeric symmetry of the *purp*-LH2 ring is octameric and that the *purp*-LH2 complexes do not form dimers in the crystal lattice in the present crystal form.

This work was supported by funds from the BBSRC. We thank the Diamond Light Source for access to beamline I02 (MX6683-2) that contributed to the results presented here. We also thank Randy Read for helpful comments.

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Figure 5

Crystal packing for the successful MR solution in space group $I4$, which forms the octameric '*purp*-LH2' ring around the fourfold axis, viewed along this axis. An *Rps. acidophila* LH2 dimer of α/β -heterodimers (represented as C α backbones in blue/green and magenta/red colours) was used as the MR model; the associated Bchl a cofactors were included in the model but are omitted here for clarity. This figure was generated in *Coot* (Emsley *et al.*, 2010).

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