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Crystallization and preliminary X-ray studies on the reaction center–light-harvesting 1 core complex from *Rhodopseudomonas viridis*

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The reaction center–light-harvesting 1 (RC–LH1) core complex is the photosynthetic apparatus in the membrane of the purple photosynthetic bacterium *Rhodopseudomonas viridis*. The RC is surrounded by an LH1 complex that is constituted of oligomers of three types of apoproteins (α, β and γ chains) with associated bacteriochlorophyll *b*s and carotenoid. It has been crystallized by the sitting-drop vapour-diffusion method. A promising crystal diffracted to beyond 8.0 Å resolution. It belonged to space group *P*1, with unit-cell parameters $a = 141.4$, $b = 136.9$, $c = 185.3$ Å, $\alpha = 104.6$, $\beta = 94.0$, $\gamma = 110.7^\circ$. A Patterson function calculated using data between 15.0 and 8.0 Å resolution suggested that the LH1 complex is distributed with quasi-16-fold rotational symmetry around the RC.

1. Introduction

In the initial step of photosynthesis in photosynthetic organisms, light energy is converted to chemical energy after the photon captured by the light-harvesting complex (LH complex) is transferred to the reaction center (RC). In the membrane of purple photosynthetic bacteria, the RC forms a supramolecular complex with the LH1 complex, which is composed of oligomers of α-helical apoproteins together with associated bacteriochlorophylls and carotenoid. Each LH apoprotein in the complex has a single transmembrane helix and binds to the bacteriochlorophyll and carotenoid noncovalently (Cogdell *et al.*, 1999). Electron-microscopic and atomic force microscopic studies of complexes from several photosynthetic bacteria have shown that the LH1 complex forms a ring around the RC (Miller, 1982; Ikeda-Yamasaki *et al.*, 1998; Walz *et al.*, 1998; Jamieson *et al.*, 2002; Siebert *et al.*, 2004; Fotiadis *et al.*, 2004). The 4.8 Å crystal structure from *Rhodopseudomonas palustris* (Roszak *et al.*, 2003) indicated that the RC is enclosed by 15 pairs of LH1 apoproteins (α- and β-chains) and an additional single transmembrane helix, making an incompletely closed ring. Although the major photosynthetic bacteria express different types of LH complexes that are oligomers of α- and β-apoproteins, *R. viridis* expresses a LH1 complex that is an oligomer of α-, β- and γ-apoproteins (Brunisholz *et al.*, 1985; Michel *et al.*, 1986). Despite many structural studies, the structure of the RC–LH1 core complex has not been sufficiently characterized to elucidate the energy-transfer and photochemical reaction processes in the photosynthetic apparatus. The present paper describes the crystallization and preliminary X-ray studies of the RC–LH1 core complex from *R. viridis* in order to elucidate its structure.

2. Experimental

2.1. Protein purification

The RC–LH1 core complex from isolated *R. viridis* (ATCC19567) chromatophores was solubilized with 5%(*w*/*v*) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, Dojindo). After purification by polyacrylamide gel electrophoresis with 1%(*w*/*v*) CHAPS (Hara *et al.*, 1990), the complex was subjected to Sepharose CL-2B size-exclusion chromatography (Amersham Biosciences) with a pH 8.0 buffer solution of 10 m*M* Tris–HCl (Sigma), 5%(*w*/*v*) glycerol (Wako) and 0.5%(*w*/*v*) CHAPS. Sedimentation-

velocity measurements were carried out for the collected fractions with $A_{280}/A_{1020} \leq 0.9$ (Odahara, 2004) in a Beckman–Coulter XL-I analytical ultracentrifuge. The velocity data were analyzed by direct modelling of the sedimentation boundary by finite-element solutions of the Lamm equation using the software *SEDFIT* to obtain $c(s)$, the distribution of sedimentation coefficients (Schuck, 2000). The results indicated that the main species with a sedimentation coefficient of 15.68 Sv (1 svedberg = 10^{-13} s) is populated by about 93.2% of the total mass of solutes and is practically homogeneous. Adjusting the concentration of the protein, the solution was used for crystallization after it had been determined that purified solutions do not entrap other peptides using SDS–PAGE (ATTO Corporation, PAGEL AE-6000).

2.2. Crystallization and collection of X-ray diffraction data

Prior to crystallization, the detergent in the solution was exchanged from CHAPS to decyl-β-D-maltopyranoside (DM, Calbiochem) following the discussions of Külbrandt (1998) and Odahara (2004). The protein solution was washed three times with 0.5%(*w*/*v*) DM and subsequently washed six times with 0.1%(*w*/*v*) DM using an ultrafiltration apparatus employing a polysulfone membrane (Advantec, UK-200) with a molecular-weight cutoff of 200 kDa. In this process, the new detergent was concentrated so that it remained above the critical micelle concentration [CMC; 0.087%(*w*/*v*) for DM] even if diluted (Odahara, 2004).

The concentration of the RC–LH1 core complex was calculated from its measured optical density using a molar extinction coefficient of 4.3 μM^{-1} cm^{-1} at 1020 nm (Hara *et al.*, 1990). Crystallization was performed with the sitting-drop vapour-diffusion method (Ducruix & Giegé, 1992). 2 µl concentrated sample was equilibrated against 500 µl reservoir solution at 278 K after mixing with the same volume of reservoir solution. The initial conditions for screening were determined by the method established by Odahara (2004). Combinations of PEG 4000 and various salts were initially used. As a result, a shower of tiny crystals formed in the presence of Li_2SO_4, sodium acetate, $(NH_4)_2SO_4$, $MgCl_2$, $NiCl_2$, $NiSO_4$, zinc acetate and $ZnSO_4$. The observed crystallization mainly depended on the cation type, which is consistent with the phenomenon that the effect of salts on protein solubilities, when used together with PEG as precipitants, is dominated by cations rather than anions. Attempts to improve the size and quality of the obtained crystals took place using a mixture of 0.1%(*w*/*v*) DM and 1.0%(*w*/*v*) *n*-octyl-β-D-maltopyranoside (OM, Anatrace) instead of 0.1%(*w*/*v*) DM, because OM, which has the smaller alkyl tail, modifies the structure and size of the micelle and hence may improve the interactions among lattices. PEG 2000 was used instead of PEG 4000 and the salt concentration was halved in order to enhance the attractive electrostatic forces between proteins. After several rounds of optimization, thin plate-shaped dark green crystals grew in one to two weeks from 26 μM complex solution,

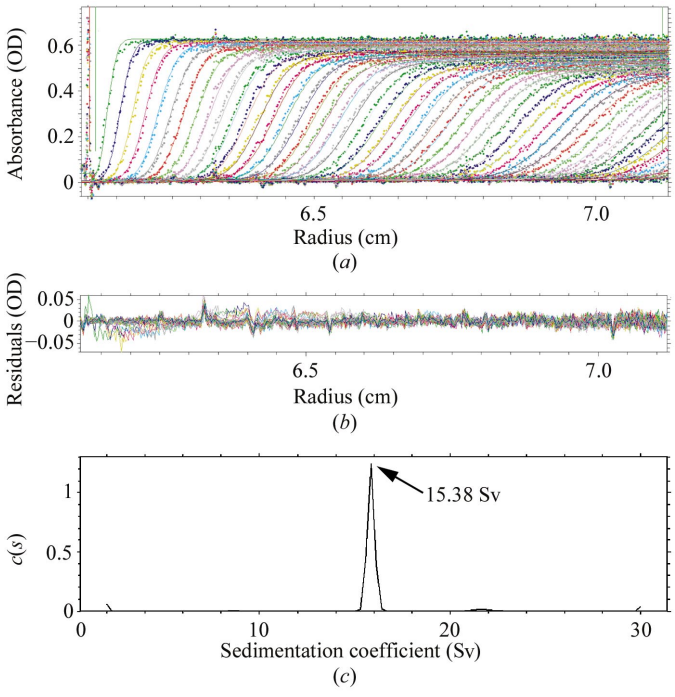

Figure 1
Sedimentation-velocity data and the sedimentation coefficient. (*a*) Raw sedimentation-velocity data. Experimental absorbance profile (circles) obtained at a rotor speed of 40 000 rev min^{-1} at 293 K in 50 m*M* Tris–HCl pH 8.0, 0.1 *M* NaCl, 0.1%(*w*/*v*) DM at RC–LH1 core complex concentrations of 0.18 µ*M* are shown. The solid lines show the best-fit sedimentation distributions. (*b*) Residuals of the fits corresponding to the data. (*c*) Distribution of the sedimentation coefficient. The distribution of the sedimentation coefficient was calculated with the Lamm equation modelling from the observed sedimentation velocity.

Table 1
Data-collection statistics.

X-ray source	SPring-8 BL26B1
Wavelength (Å)	1.000
Maximum resolution (Å)	8.0
Crystal-to-detector distance (mm)	300
Oscillation angle (°)	1
No. frames	180
Exposure time (s)	20
No. crystals	1

Table 2
Data-processing statistics.

Values in parentheses are for the outer shell.

Space group	*P*1
Unit-cell parameters	
a (Å)	141.4
b (Å)	136.9
c (Å)	185.3
α (°)	104.6
β (°)	94.0
γ (°)	110.7
Resolution range (Å)	48.50–8.00 (8.28–8.00)
No. observed reflections	23060 (2396)
No. unique reflections	12489 (1274)
Average redundancy	1.85 (1.88)
Completeness (%)	96.1 (98.5)
$R_{\rm merge}$†	0.105 (0.356)
$\langle I/\sigma(I)\rangle$	3.5 (1.4)

† $R_{\rm merge} = \sum_{\mathbf{h}}\sum_i I_i(\mathbf{h}) - \langle I(\mathbf{h})\rangle / \sum_{\mathbf{h}}\sum_i I_i(\mathbf{h})$, where I_i is the ith measurement of reflection **h** and $\langle I(\mathbf{h})\rangle$ is a weighted mean of all measurements of **h**.

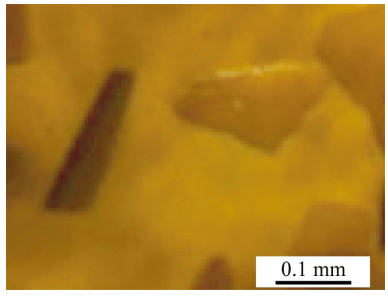

Figure 2
Crystals of *R. viridis* RC–LH1 core complex.

10 m*M* Tris–HCl pH 8.0, 1.0%(*w*/*v*) OM and 0.1%(*w*/*v*) DM using a reservoir solution consisting of 50 m*M* Tris–HCl pH 8.0, 13%(*w*/*v*) PEG 2000 and 30 m*M* $MgCl_2$. Typical dimensions of the crystals were 0.25 × 0.15 × 0.02 mm (Fig. 2).

X-ray diffraction data were collected using a Weissenberg camera for macromolecules (Sakabe, 1991) at Photon Factory (Tsukuba, Japan), a MAR CCD detector at BL41XU (Kawamoto *et al.*, 2001) and a Rigaku/MSC Jupiter 210 detector at BL26B1 (Yamamoto *et al.*, 2002) at SPring-8 (Harima, Japan). The crystal was soaked with a cryoprotectant solution consisting of 50 m*M* Tris–HCl pH 8.0, 20%(*w*/*v*) PEG 2000, 30 m*M* $MgCl_2$, 1.0%(*w*/*v*) OM, 0.1%(*w*/*v*) DM and 25%(*w*/*v*) glycerol for several minutes and flash-frozen using cold nitrogen gas in order to maintain the temperature at 100 K. The collected data were integrated and scaled using the *d*TREK* data-processing package (Pflugrath, 1999) from the *CrystalClear* suite (Rigaku/MSC). Data-collection parameters and data-processing statistics are summarized in Tables 1 and 2.

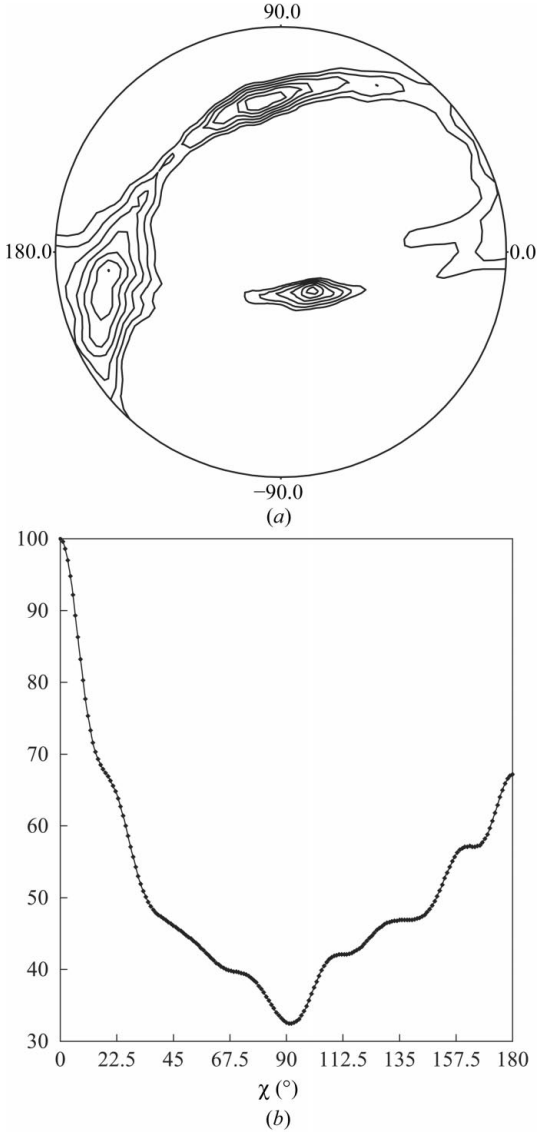

Figure 3
(*a*) $\chi = 180^\circ$ section of the self-rotation function calculated from the data set from a *R. viridis* RC–LH1 core complex crystal. The resolution of the data used was 15.0–8.0 Å. The integration radius was 15–8.0 Å and the orthogonal *x*, *y* and *z* axes are along the *a*, *c** × *a* and *c** axes, respectively. (*b*) Polar distribution about an axis of (154°, 132°, χ) for vectors centred at the origin (self-rotation function).

3. Results

The triclinic crystal diffracts to 8.0 Å. After the X-ray measurements, it was shown by SDS–PAGE analysis that the crystal used for X-ray measurements was composed of both RC and LH1 proteins (data not shown). The molecular weight was calculated to be 441 kDa by assuming the RC–LH1 core complex to be composed of one RC (141 kDa) and 16 LH1 (6.8 kDa α-apoprotein, 6.1 kDa β-apoprotein, 4.0 kDa γ-apoprotein and two 0.9 kDa bacteriochlorophyll *b*) proteins. The number of RC–LH1 core complexes per asymmetric unit (*Z*) was estimated to be either two, three or four, from which the corresponding V_M was calculated to be 3.62, 2.42 or 1.81 $Å^3\ Da^{-1}$, respectively (Matthews, 1968).

To evaluate the local symmetry of the RC–LH1 core complexes, the self-rotation function was calculated between 15.0 and 8.0 Å, applying the integration radius of 50 Å by setting NCODE as 1 (orthogonal *x*, *y* and *z* axes are along *a*, *c** × *a* and *c** axis, respectively). The program *POLARRFN* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) was utilized for the calculation. The correlation function plotted against the χ axis gave peaks at $\chi = 22.5$, 45 and 180° at $\omega = 154^\circ$ and $\varphi = 132^\circ$ (Figs. 3*a* and 3*b*). The prominent peak at $\chi = 180^\circ$ indicated that the RC possesses pseudo-local twofold symmetry. Weak and ambiguous peaks at $\chi = 67.5$ and 90° suggests that the complex has a quasi-16-fold symmetry. Presumably, the RC–LH1 core complex contains a ring of 16 LH1 proteins (Scheuring *et al.*, 2003), as shown in the structure of *R. palustris* (Roszak *et al.*, 2003). Although the *R. palustris* RC–LH1 core complex contains α- and β-apoproteins, the LH1 protein of *R. viridis* contains three types of apoprotein (α, β and γ; Brunisholz *et al.*, 1985); the three types of apoprotein seem to make similar quasi-16-fold symmetrical rings as found in the *R. palustris* RC–LH1 core complex [$\alpha_{15}\beta_{15}$ and helix W (unknown sequence)].

In this paper, we have reported the production of three-dimensional crystals of the RC–LH1 core complex from *R. viridis*. The results of self-rotation function calculations suggest that the LH1 complex has a quasi-16-fold symmetry around RC. Determination of the orientation and position of the molecules involved and further search trials for obtaining crystals that will diffract to high resolution are now in progress.

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