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# Preliminary X-ray crystallographic studies of photosynthetic reaction center from a thermophilic sulfur bacterium, *Chromatium tepidum*

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**Abstract**

A membrane protein complex, photosynthetic reaction center purified from the thermophilic purple sulfur bacterium, *Chromatium tepidum* has been crystallized from a phosphate buffer containing a detergent, *n*-octyl-β-D-glucopyranoside and a precipitant, polyethylene glycol 4000. The crystals diffracted X-rays beyond 3Å resolution with synchrotron radiation and are suitable for high-resolution X-ray crystallographic studies. The crystals belong to the orthorhombic space group $P2_12_12_1$ with unit-cell dimensions of $a = 136$Å, $b = 197$Å, and $c = 82$Å. Assuming that they contain one reaction center complex in the asymmetric unit, $V_M$ was calculated to be 4.3 Å$^3$/Da, which agrees with the values obtained in the membrane protein complexes.

*Key words:* Membrane protein; Reaction center; Photosynthesis; Crystallization; X-ray structure analysis

## 1. Introduction

The primary processes in photosynthesis involve absorption of light energy and its conversion into chemical energy. The reaction centers (RCs) from purple photosynthetic bacteria are integral membrane proteins that accept energy from light-harvesting pigment protein complexes and perform the initial charge separation. They have many prosthetic groups, bacteriochlorophyll dimer ($(BChl)_2$, special pair), bacteriochlorophyll monomer (accessory BChl), bacteriopheophytin (BPh) and quinone, through which electrons are transferred (reviewed in [1]). Three-dimensional structures of RCs from two purple non-sulfur bacteria, *Rhodopseudomonas (Rps.) viridis* and *Rhodobacter (Rb.) sphaeroides* have been reported [2–4] (reviewed in [5]). They have two hydrophobic transmembrane L and M subunits and a semi-hydrophilic H subunit. In addition, a tightly bound cytochrome subunit is also included in the case of *Rps. viridis*.

Both *Rps. viridis* and *Rb. sphaeroides* above mentioned are mesophilic and non-sulfer bacteria which optimally grow at a temperature around 30°C. On the other hand, *Chromatium (C.) tepidum* is a thermophilic and sulfur purple bacterium with an optimum growth temperature of 48–50°C [6]. This bacterium contains light-harvesting complexes B917 and B800–855 and a RC as photosynthetic apparatus [7]. Intact chromatophores are stable even up to 70°C [8]. The RC from *C. tepidum* contains also a bound *c*-type cytochrome subunit (44 kDa) in addition to L, M and H protein subunits (25, 30 and 34 kDa) [7]. The RC shows a thermal stability up to 47°C in detergent solutions as revealed from absorption and circular dichroism spectra [9] and is expected to have some characteristic structural differences, especially in some specific roles of the amino acids, compared with those from mesophilic bacteria. The three-dimensional structure of this RC is indispensable to clarify the structural specificity which induces thermostable natures of the protein. It has been reported that the RC from *C. tepidum* could be highly purified, enough to be crystallized by the use of polyethylene glycol (PEG) 4000 as a precipitant [10]. Here, we report the crystallization of this RC suitable for high-resolution X-ray diffraction works and its preliminary crystallographic characterization.

## 2. Experimental

### 2.1. Crystallization

The purification procedure of RCs from *C. tepidum* was described previously [7,8,10]. For further purification, RCs in 0.05%LDAO (*N*,*N*-dimethyldodecyl-amine-*N*-oxide)/10 mM Tris-HCl buffer (pH 8.0) were applied onto a DEAE-Toyopearl column, which was previously equilibrated with a 15 mM phosphate buffer (pH 7.0). The detergent-exchanged RCs were then obtained by elution with 60 mM NaCl/0.8% *n*-octyl-β-D-glucopyranoside/15 mM phosphate buffer (pH 7.0).

Protein solutions at concentrations of 20–40 mg/ml were prepared in the presence of 4–7% (w/v) PEG 4000 as a precipitant in a 15 mM phosphate buffer (pH 7.0) together with 0.36 M NaCl, 0.1% (w/v) $NaN_3$, and 0.1 mM EDTA(ethylenediamine tetraacetic acid). Droplets (10–20 μl) of the protein solutions were pipetted onto glass slides for the sitting drop vapor-diffusion procedure and then vapor-equilibrated with 1ml of reservoir solutions in which concentrations of the precipitants were 20–40% higher than those of the protein solutions. Crystallizations were carried out at 4°C in the dark.

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*2.2. X-ray diffraction study*

The crystals were mounted in glass-capillaries with a trace amount of the mother liquor. The precession photographs were taken at room temperature with a Huber precession/rotation camera using graphite-monochromatized Cu-$K\alpha$ radiation generated by a rotating anode generator (MAC Science M18X) operated at 50 kV and 90 mA. Photographic works were also performed with synchrotron radiation at 2.5 GeV at the BL-6$A_2$ beam line of the Photon Factory, the National Laboratory for High Energy Physics, Japan. The X-ray beam was monochromatized by the Si(111) monochromator system. The wavelength used was 1.00 Å A screenless Weissenberg camera was used with a 0.1-mm aperture collimator and a cylindrical cassette of 429.7 mm radius [11]. The diffraction intensities were recorded on a 200 × 400 mm imaging plate (Fuji Photo Film Co. Ltd.). The plates were digitized at 100 mm intervals on a Fujix BA100 read-out system (Fuji Photo Film Co. Ltd.) [12]. The intensity data were evaluated using the program system WEIS [13] and processed with program package PROTEIN [14].

## 3. Results and discussion

Within one to eight weeks, well-shaped RC crystals with average dimensions of 0.1 mm × 0.1 mm × 0.25 mm were obtained (Fig. 1). The whole components of protein subunits (L, M, H and cytochrome subunits) in these crystals were confirmed by SDS-PAGE.

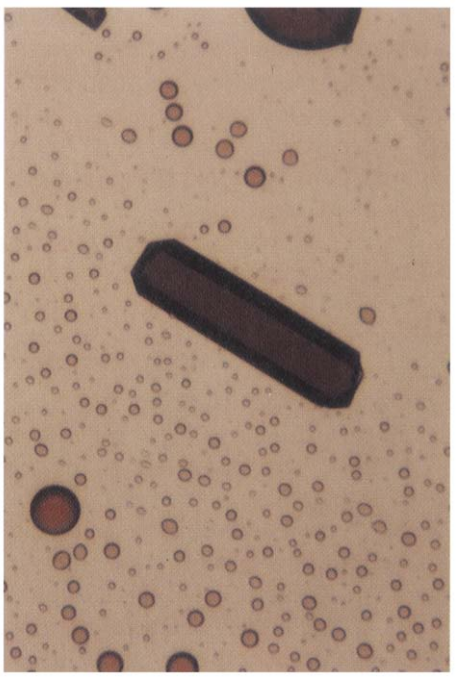

Fig. 1. Crystals of reaction center from *Chromatium tepidum* suitable for X-ray diffraction work.

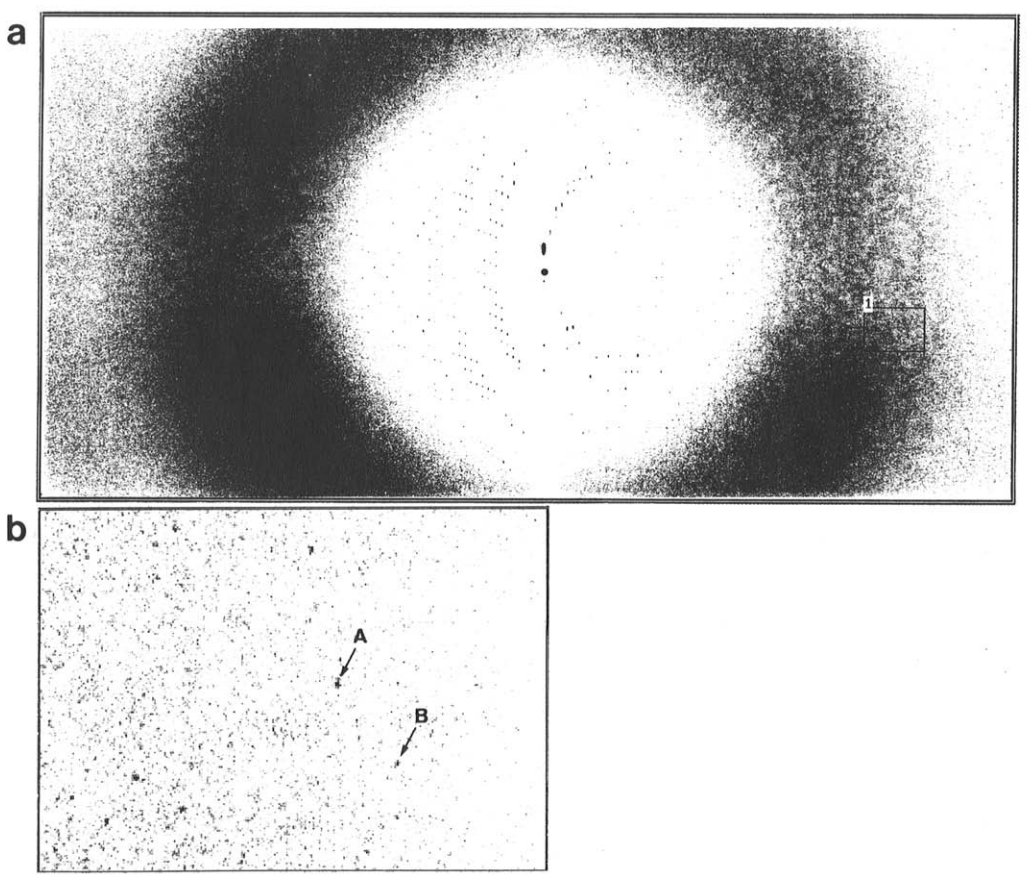


Fig. 2. An image pattern of a Weissenberg photograph recorded on an imaging plate taken with synchrotron radiation (see section 2). (a). A full pattern of the 200 × 400 mm imaging plate where reflections within the rotation range of 4.0° are recorded with the c* axis rotation. The exposure time was 120 s. The central black circle indicates the direct beam position. Both left and right edges correspond to about 2.2 Å resolution. (b). A magnified pattern of the square area (indicated as 1) in (a). Both left and right edges correspond to about 3.3 Å and 2.8 Å resolution, respectively. The arrows A and B shows reflection spots corresponding to about 3.0 Å and 2.9 Å resolution, respectively.

Diffraction patterns in precession and oscillation photographs showed that the crystals belong to the orthorhombic system. A Weissenberg photograph taken with synchrotron radiation is presented in Fig. 2. The crystals diffracted X-rays beyond 3 Å resolution. The systematic absences of reflections indicated that the space group is $P2_12_12_1$. The unit-cell dimensions were determined to be $a$ = 136 Å, $b$ = 197 Å, and $c$ = 82 Å, with the unit-cell volume of $2.19 \times 10^6$ Å$^3$. Assuming one RC molecule per asymmetric unit and the estimated molecular mass of 133 kDa, the crystal volume per unit molecular weight, $V_M$ [15], was calculated to be 4.3 Å$^3$/Da. This value agrees with that obtained in the RC from *Rps. viridis* of 4.9 Å$^3$/Da [16]. They are the common values for integral membrane proteins, which correspond to the solvent content of more than 70% in the crystal, if the volume of disordered detergent molecules is ignored. Intensity data were collected on a screenless Weissenberg camera and imaging plates with synchrotron radiation. The present crystal was suitable for the high-resolution structure analysis, but was not so stable to X-ray irradiation to take complete data set with one crystal. The evaluated and merged data set with 17,724 unique reflections included 44% of theoretically calculated number of reflections from 10 to 3.0Å resolutions, where the $R_{merge}$ value was 0.095 ($R_{merge} = \Sigma_h\Sigma_i|I(h)_i - < I(h) >|/\Sigma_h\Sigma_i I(h)_i$ where $I(h)_i$ is the $i$th measurement and $<I(h)>$ is the mean of all measurements of $I(h)$).

The data collection to obtain the complete data set is in progress, which is expected to result in the achievement of the structure analysis by the Patterson search method based on the molecular model of the RC from *Rps. viridis*.

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