FOR THE RECORD

Crystallization of two integral membrane pigment–protein complexes from the purple-sulfur bacterium *Chromatium purpuratum*

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Purple photosynthetic bacteria are divided into two groups based on their ability or inability to use reduced sulfur compounds as the primary electron donor in photosynthesis. Biochemically the purple-nonsulfur organisms (such as members of the genera *Rhodopseudomonas* and *Rhodobacter*) are relatively well characterized. The purple-sulfur bacteria (members of the Chromatiaceae and the Ectothiorhodospiraceae) are thought to be more ancient than the purple-nonsulfur organisms. The photosynthetic apparatus of all purple bacteria consists of a reaction center (RC) and one to three light-harvesting complexes. The RC is the site of transmembrane charge separation. In its isolated form it is composed of three integral membrane proteins (H, M, and L) and their associated cofactors, and in some organisms there is a fourth subunit, a tetraheme *c*-type cytochrome. The RC is surrounded by light-harvesting complexes that funnel captured energy to the RC. All purple photosynthetic bacteria possess the light-harvesting complex 1 (LH1), which surrounds the RC. The two polypeptides of this complex, known as α and β , are encoded by the same operon that encodes the M and L subunits (Youvan et al., 1984; Zhu & Kaplan, 1985) and are present in a fixed stoichiometry to the RC (Aagard & Sistrom, 1972). Surrounding the RC-LH1 (also known as the core) in most photosynthetic purple bacteria is a second antenna system, called the peripheral antenna or LH2. Unlike LH1, the amount of LH2 present varies with the environmental conditions of growth: for example, some organisms increase the expression of LH2 to compensate for growth at diminished light intensity (reviewed in Hawthornethwaite & Cogdell [1991]

and Zuber & Brunisholz [1991]). The basic structure of all LHs is thought to be a heterodimer of the small α and β polypeptides (5–10 kDa) that bind pigments in a specific stoichiometry distinctive of LH1 or LH2. Sequence analysis and circular dichroism measurements of antenna complexes of purple-nonsulfur bacteria suggest that each LH1 or LH2 antenna polypeptide spans the membrane once in the form of an α -helix (Cogdell & Scheer, 1985; Brunisholz et al., 1986). The limited biochemical data on purple-sulfur organisms (members of the Chromatiaceae) indicate subunit homology at the level of function and of primary sequence (Bissig et al., 1990; Kerfeld et al., manuscripts submitted and in prep.).

Attempts to crystallize integral membrane pigment–protein complexes have focused exclusively on those derived from purple-nonsulfur bacteria. There have been numerous reports of crystallization of peripheral antenna complexes from a variety of purple-nonsulfur organisms (Cogdell et al., 1985; Welte et al., 1985; Hawthornethwaite et al., 1989; Michel, 1990; Guthrie et al., 1992; Nunn et al., 1992). The crystal structure of the RC from *Rhodopseudomonas viridis* was the first integral membrane protein structure solved at atomic resolution (Deisenhofer et al., 1985) followed by the RC from *Rhodobacter sphaeroides* (Allen et al., 1986; Chang et al., 1986). These structures were quite similar to one another, making it unclear as to whether certain RC structural features that are conserved between the two organisms are the result of functional constraints or reflect a close evolutionary relationship.

Chromatium purpuratum is a marine purple-sulfur organism first described by Imhoff and Truper in 1980. The photosynthetic apparatus of this organism can be fractionated by Deriphat polyacrylamide gel electrophoresis (Ferguson et al., 1991) after detergent solubilization into

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three complexes: the LH2, a carotenoid-protein complex, and the RC-LH1 (core). Here we report the crystallization of two of these integral membrane pigment-protein complexes.

Results

The core

The RC of *C. purpuratum* is composed of H (30 kDa), M (27 kDa), and L (19 kDa) subunits and a tetraheme cytochrome (39 kDa) that is retained during purification. The purified *Rps. viridis* RC also contains this subunit (Thorner et al., 1969), and the crystal structure of this complex revealed that it was tightly bound by a single lipid moiety (Deisenhofer & Michel, 1989), whereas this subunit is easily dissociated from the RCs of *Chromatium minutissimum* (Zakharova et al., 1992) and *Rhodocyclus gelatinosus* (Agalidis & Reiss-Husson, 1992). The calculated molecular weight for an isolated *C. purpuratum* RC (assuming a 1:1 stoichiometry of all subunits) is approximately 120 kDa. In addition, in *C. purpuratum*, the LH1 remains associated with the RC; in most purple bacteria, purifications yield isolated RCs and LH1s (summarized in Hawthornethwaite & Cogdell [1991]). For each RC an LH1 ($(\alpha\beta)_6$ or an $(\alpha\beta)_{12}$ is associated (Varga & Staehlin, 1985; Nunn et al., 1992), providing an additional molecular mass of approximately 60 or 120 kDa. The molecular weight of the RC-LH1 as estimated from size-exclusion high-performance liquid chromatography in the presence of detergent is approximately 550 kDa (Kerfeld et al., 1992). This is larger than expected for an association of a single RC and LH1. It has been suggested that the bacterial RC exists as a multimer in vivo (Joliet et al., 1990). Thus the large molecular weight estimated for the undissociated *C. purpuratum* RC-LH1 may indicate that there is more than one RC incorporated in this basic photosynthetic unit. An atomic resolution structure of the intact core complex will allow us to address this possibility. Moreover it will provide insight into how energy is transferred from the LH1 to the RC and, by comparison with the two crystal structures of the RCs from purple-nonsulfur organisms, may allow a distinction to be made between structural features that are conserved due to function or to evolutionary relatedness.

Initially the core was crystallized in hanging drops at room temperature containing 3 mg/mL protein, 0.03% dodecyl maltoside, 20 mM CHES (2-[N-cyclohexylamino]-ethanesulfonic acid), pH 9.5, 2% polyethylene glycol (PEG) 3350, 0.1 M LiCl, and 0.002% hexanetriol, equilibrated with a reservoir containing 10% PEG 3350, 100 mM CHES, pH 9.5, and 0.5 M LiCl. Typically, single crystals appeared in 2–3 weeks following a phase separation. This extended period of time necessary for crystal growth is often characteristic of membrane proteins. We sought to expedite the crystal growth process to facilitate

the screening of a large number of conditions. The addition of ethanol at concentrations between 5% and 20% accomplished this; single crystals now appeared within 2–3 days and continued to grow for approximately 1 week. The core crystal shown in Figure 1A appeared after 3 days in the aforementioned conditions with the addition of 10% ethanol to the reservoir and 2% ethanol to the protein drop. Addition of ethanol did not exacerbate protein denaturation: the pigmentation and amount of precipitated protein was similar in protein drops with and without added ethanol. Further experiments have shown that methanol and acetone serve a similar function. Most likely the depression of the dielectric constant of the mother liquor by the organic solvent is responsible for the faster rate of crystallization. In addition these additives may be acting as small amphiphiles, altering the critical micelle concentration of the detergent and perhaps the shape and size of the micelle.

Crystals similar to that shown in Figure 1A, analyzed with a Rigaku RAXIS II imaging plate equipped with an RU-200 rotating anode generator, have shown limited diffraction to 7 Å and are fairly radiation tolerant. We have not yet been able to determine the space group with confidence, but 2° oscillation images revealed a centered rectangular net with $a^* = 0.0087 \text{ Å}^{-1}$, $b^* = 0.0041 \text{ Å}^{-1}$, $\gamma = 90^\circ$. Based on spacings in the perpendicular direction, c is approximately 350 Å, giving a primitive unit cell volume of $5 \times 10^6 \text{ Å}^3$. The unit cell is consistent with C2 as the lowest symmetry space group; higher symmetry elements do not appear to be present. Assuming space group C2 with four 550-kDa complexes in the centered cell ($V = 10^7 \text{ Å}^3$), the V_m value (Matthews, 1968) would be 4.5. Although this is in the upper part of the accepted range, other membrane protein crystals also tend to exhibit unusually high values: porin, 2.9–5.2 (Pauptit et al., 1991); *Rps. viridis* RC, 5.24; and *Rb sphaeroides* RC, 4.1.

Fig. 1. **A:** Crystal of the RC-LH1 complex from *C. purpuratum*; approximately $150 \times 100 \times 10 \mu\text{m}$. **B:** Crystals of *C. purpuratum* LH2; the longest dimension is approximately $200 \mu\text{m}$.

The peripheral antenna

The LH2 of *C. purpuratum* has somewhat unusual spectral characteristics (Cogdell et al., 1990) and refinement of denaturing electrophoresis conditions revealed that the *C. purpuratum* LH2 is composed of six low-molecular-weight (<10 kDa) subunits. This composition is invariant in organisms grown under different environmental conditions. In addition, N-terminal sequence analysis of these subunits indicates that each is unique and that their N-termini, preceding the presumed membrane span, is among the longest shown thus far for LH2 proteins; this may facilitate crystallization of this complex. Sequence analysis and circular dichroism measurements suggest the presence of some β -type secondary structure (Kerfeld et al., manuscript submitted). This differs from what is expected for antenna complexes of purple-nonsulfur organisms. The crystals shown (Fig. 1B) were grown from a hanging drop containing 6.2 mg/mL purified LH2, 50 mM Tris, pH 7.0, 0.6% octyl glucoside, 11.5% PEG 3350, 125 mM NaCl, and 0.01% hexanetriol equilibrated over a reservoir containing twice the concentration of buffer and precipitants in the protein drop. Crystals appeared after 6 weeks at room temperature; these crystals diffract weakly. Addition of ethanol to this and similar crystallization conditions results in a shower of small, birefringent clusters of crystals overnight; excessive nucleation has continually been a problem with this complex with or without added organic solvent.

Conclusions

For *C. purpuratum* pigment-protein complexes the addition of organic solvents assists the rapid screening of crystallization conditions; this approach may be applicable to detergent-membrane protein crystallization systems in general. Although we have yet to obtain high-resolution diffraction from crystals of these purple-sulfur bacterial pigment-protein complexes, we have shown that they are amenable to crystallization. Obtaining the atomic structure of both the peripheral antenna and the RC-LH1 from a single organism would make a more complete understanding of the energy transfer events in bacterial photosynthesis possible.

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