Two-dimensional crystallization of reaction centers from *Chloroflexus aurantiacus*

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Two-dimensional crystals of photosynthetic reaction centers from *Chloroflexus aurantiacus* were obtained from protein-lipid-detergent micelles by detergent dialysis. The size of crystals was up to 2 μm. Some of them were multilayered crystals. However, other crystal forms were also observed. Preliminary image processing analysis showed that crystals of one crystal form referred to two-sided plane group p2 and had the following unit cell parameters: $a=17{,}6$ nm, $b=18{,}0$ nm, $\gamma=84°$. The contour map of the crystal stain-excluding region was calculated by the Fourier-filtering procedure at about 2 nm resolution.

Reaction center; Two-dimensional crystal; Electron microscopy; Image processing

1. INTRODUCTION

Primary photochemical reactions in bacterial photosynthesis are known to occur in integral membrane protein systems, i.e. reaction centers (RC). Although the detailed mechanisms of the functioning of these multimolecular systems are still obscure, the studies of the structural organization of the photosynthetic systems at all levels are important. The membrane organization of the bacteria RC is under extensive study. The structure of the RC molecules of purple bacteria *Rhodopseudomonas viridis* [1,2] and *Rhodopseudomonas sphaeroids* [3,4] were determined at atomic resolution by X-ray analysis. The spatial structure of the photosynthetic RC of green bacteria *Chloroflexus aurantiacus* in the membrane is as yet unknown. Staehelin with coworkers analyzed a general morphology of *Chloroflexus aurantiacus* bacteria [5]. Recently primary structures of the L- and M-subunits of *Chloroflexus* RC were established, that allowed modeling the topography of the polypeptide chains in the membrane [6,7].

The structural organization of membrane proteins can be studied by electron microscopy of two-dimensional crystals followed by computer image reconstruction techniques [8]. The present work was aimed at two-dimensional crystallization of *Chloroflexus* RC and preliminary characterization of reconstituted structures.

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Abbreviations: LDAO, lauryldimethylamine N-oxide; RC, reaction center

2. MATERIALS AND METHODS

Isolation and purification of RC, determination of spectral and other chemical parameters were carried out as described in [4,5]. Lecithin from soybean (Sigma) was solubilized by lauryldimethylamine *N*-oxide (LDAO). For reconstitution experiments, solubilized lecithin was added to the RC sample (10 mg/ml of protein in 50 mM of Tris-HCl, pH 9.0, 0.1% LDAO) at various proportions. Then detergent was removed by dialysis under various conditions (temperature, dialysis rate, ionic strength, ion composition of dialysis media) [8].

Electron microscopy studies, optical diffraction and image processing were done as described in [10].

3. RESULTS AND DISCUSSION

Typical electron micrographs of RC crystals are presented in Fig. 1 A,B,C. The maximum size of the crystals is about 2 microns. Some structures are the multilayered two-dimensional crystals (Fig. 1B). This fact complicates the crystal structure analysis. Nevertheless about 20% of crystal structures are not multilayers (Fig. 1A). The relative amount of noncrystal material (liposomes, amorphous precipitate, etc.) in the samples is about 30%. Sometimes more than one different crystalline pattern was located within the membrane (e.g. see Fig. 1C). Therefore there exist different crystal forms. Structural studies of crystal forms is in progress at present. In this paper we analyzed crystalline areas of only one crystal form (squared in Fig. 1C).

The optical diffraction and Fourier analysis of such

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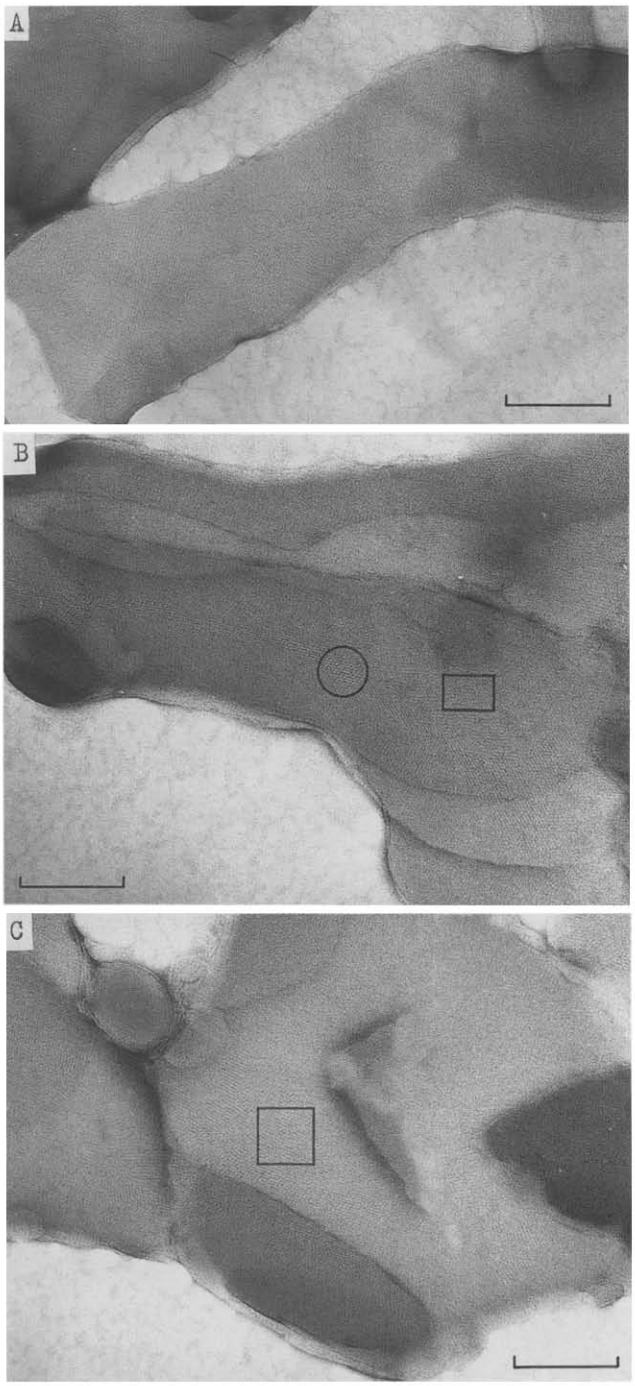

Fig. 1. (A, B′, C″) Electron micrographs of two-dimensional crystals of *Chloroflexus aurantiacus* reaction centers. Negative staining by 2% aqueous solution of uranyl acetate. Bar represents 0.2 μm. In (B) the circled and rectangular areas represent different crystal forms. In (C) the squared area represents a crystal form selected for image reconstruction.

crystal images showed the resolution range to be about 2–3 nm. A unit cell has the following parameters: $a = 17{,}6$ nm, $b = 18{,}0$ nm, $\gamma = 84°$. The values of phases of main reflections were about 0° or 180° after origin refinement procedure (best phase residual was about

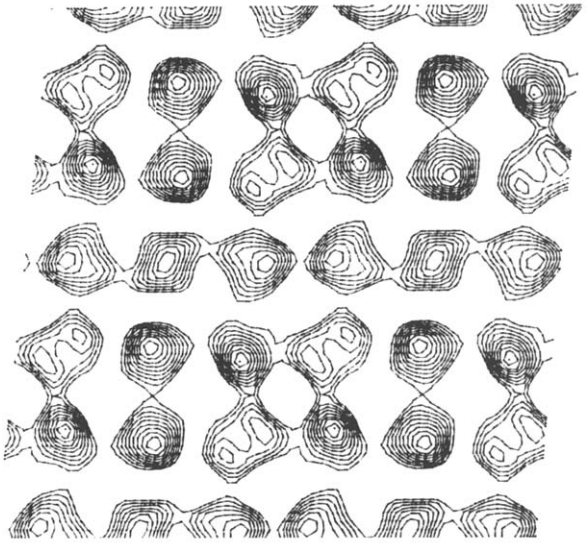

Fig. 2. Contour map of stain-excluding region of two-dimensional *Chloroflexus* RC crystal (4 unit cells).

18°). For these reasons such crystals can be referred to as two-sided plane group p2 [11]. Fig. 2 presents a contour map of Fourier-filtered image of RC two-dimensional crystal (4 unit cells). Note that there are 9 stain-excluding regions per unit cell.

The comparison of amino acid sequences of L- and M-subunits of *Chl. aurantiacus* and purple bacteria [6,7] indicates a high extent of structural homology. According to X-ray data, the purple bacteria heterodimer structure, which consists of L- and M-subunits, is an elliptical cylinder [4]. The height of the cylinder is approximately 5 nm, and the long and short diameters of the elliptical cross-section are 6 and 4 nm, respectively. The comparison of the square of elliptical cross-section of heterodimer with the square of RC crystal unit cell shows that a single unit cell corresponds to maximum 12 L, M-heterodimers. Due to the fact that there are 9 stain-excluding regions per unit cell it is more likely that a single unit cell corresponds to 9 L, M-heterodimers. The final result might provide the three-dimensional image reconstruction.

Thus, electron microscopic studies of two-dimensional crystals of *Chloroflexus* RC gives an opportunity to get information on the spatial organization of RC in the membrane.

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