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Crystallization of photosynthetic reaction centres from *Chloroflexus aurantiacus*

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The reaction center of *Chloroflexus aurantiacus* was crystallized in the presence of detergents. The crystals were obtained using polyethylene glycol as a precipitant. Three different crystal forms were obtained; one is supposed to be hexagonal, space group $P6_322$, the second rhombic, space group $P2_122$.

Membrane protein crystallization; Reaction center; X-ray; (*Chloroflexus aurantiacus*)

1. INTRODUCTION

Photosynthetic reaction centres (Rc) are integral membrane proteins primarily involved in transformation of light energy to the energy of chemical bonds by photosynthesis in higher plants and photosynthesizing bacteria. Despite the differences, Rc in various organisms are functionally similar. This is stipulated by the general principles of their structural organization. Owing to the advances in crystallization and X-ray studies of purple bacteria Rc, the investigation of the structural basis of photosynthetic processes has become possible.

At present the Rc spatial structure from purple bacteria, *Rhodopseudomonas viridis* and *R. sphaeroides*, has been established by X-ray structural analysis [1,2]. These Rc consist of 3 polypeptide chains which are in the molecule in equimolar quantities and contain a set of chromophores and cofactors that dictate their spectral properties [3,4]. Rc of a green bacterium, *Chloroflexus aurantiacus*, have analogous spectral characteristics but are formed by two polypeptide chains. Their primary structures are homologous to the Rc L- and M-subunits of the purple bacteria [5,6]. The green bacteria Rc are considered to be the simplest today.

A study of the *C. aurantiacus* Rc crystallization conditions, and obtaining of crystals for X-ray analysis are the aims of the present study.

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2. MATERIALS AND METHODS

Rc were isolated and purified by the method described by Ovchinnikov et al. [5]. The absorption ratio for the preparations at wavelengths 280 and 806 nm was ~1.6. According to the SDS-electrophoresis in polyacrylamide gel, these Rc contained polypeptide chains of two types (28000 and 32000 Da, respectively).

The protein solubilized with lauryl dimethylamine oxide (LDAO) was concentrated to 10 mg/ml to obtain the stock solution. To change the detergent a known quantity of stock solution was dialyzed against a buffer with the required detergent in the cold for 3 days, with a 2-fold substitution of the antisolution.

Vapor diffusion and microdialysis methods were applied for crystallization. In some cases the crystal composition was tested by SDS-polyacrylamide gel electrophoresis.

The crystals were first washed out by mother liquor, then solubilized with SDS. The electrophoregrams of the samples showed two bands with mobilities typical of the Rc L- and M-polypeptides.

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3. RESULTS AND DISCUSSION

The set of conditions for the Rc crystal formation is quite wide. The major factors affecting crystallization seem to be the precipitant or detergent itself utilized for the protein solubilization, as well as the ionic strength of solutions and their respective pH. One of the classical precipitants, ammonium sulfate, turned out to be ineffective, as no crystals were observed while studying their formation conditions. The Rc crystals can be grown in the solution with polyethylene glycol (PEG). The PEG concentration necessary for the protein crystallization depends on the polymer length and temperature for solution equilibration. So, when decreasing the temperature to 4°C from 24°C the PEG concentration (M_r 4000) appropriate for crystal formation increases to 26–31%. Similar results are observed when PEG 2000 is substituted for PEG 4000.

It is important to note that the Rc crystal growth depends on ionic strength of the solution adjusted by NaCl (concentration 0.1–0.3 M) and its pH. So, in LDAO solution the crystals grow at pH 8.0–9.0. The form is hexagonal, but their maximal size is insufficient for X-ray studies. The crystals of larger size can be obtained by substituting LDAO by another non-ionic detergent, octylpolyoxyethylene (o-POE). Thus at alkaline pH in 3–4 weeks, hexagonal crystals of the size 200 × 200 × 500 μm also grow. More acidic pH (6.0–7.0) results in rhombic crystals of almost analogous sizes. Use of 1,2,3-heptanetriol as small amphiphilic molecules additive up to 3% did not affect the size or crystal ordering much either.

The preliminary X-ray studies of Rc crystals obtained in detergent o-POE at pH 8.0–9.0 showed that they belonged to hexagonal space group $P6_322$ (evidently P622) with cell parameters a = 141.9 Å, $b = c$ = 130.3 Å (fig.1). Symmetry of these spatial groups assumes the presence of the number of protein molecules in the crystal cell to be a multiple of 12. For the minimal quantity of the molecules in the cell the value of Matheus parameter [7] for the protein studied is 2.48 Å^3/Da and falls within the intervals typical of the majority of the protein crystals. The crystals grown in the same detergent at pH 6.0–7.0 are obviously a rhombic spatial group $P2_122$ with the cell parameters a = 111.0 Å, b = 116.5 Å, c = 120.0 Å (fig.1).

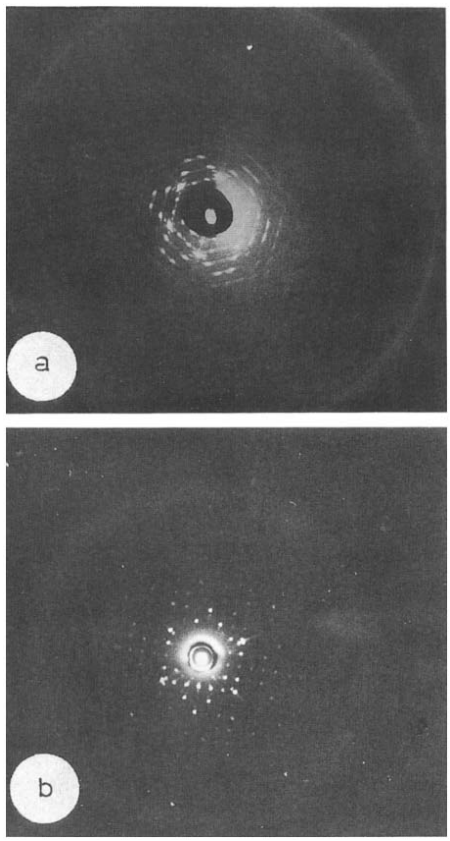

Fig.1. Precession photographs of hexagonal (a) and rhombic (b) crystal forms.

Diffraction analysis of the crystals was done using a general source of X-ray irradiation (CuK_α-line, 1200 W). Crystal life-time in an X-ray beam and resolution depends on the temperature upon exposition. Decreasing the temperature to 12°C allows registration of the reflexes in the precession photograph at 9 Å resolution. So it can be hoped that a powerful X-ray beam and further temperature decrease enhance the resolution obtainable, and provide the basis for structural research of these crystals.

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