Volume 221, number 2, 260–264 FEB 05082 September 1987

Crystallization of reaction center I of photosynthesis

Low-concentration crystallization of photoactive protein complexes from the cyanobacterium *Synechococcus* sp.

I. Witt*, H.T. Witt, S. Gerken, W. Saenger*, J.P. Dekker and M. Rögner

Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, 1000 Berlin 12 and **Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, 1000 Berlin 33, Germany*

Received 15 July 1987

The integral membrane protein complex of the photosystem I reaction center from the cyanobacterium *Synechococcus* sp. has been isolated and highly purified. At extremely low protein complex concentrations (50 μg/ml), crystallization occurred. The crystals are dichroic, photoactive and show an X-ray powder diffraction pattern.

Photosystem I; Reaction center; Membrane protein; Crystallization; (*Synechococcus* sp.)

1. INTRODUCTION

Crystallization of a photosynthetic reaction center was first reported by Michel [1] using the purple bacterium *Rhodopseudomonas viridis*. With the obtained crystals a detailed picture of the structure and organization of the proteins and pigments at 3 Å resolution could be derived [2]. In the photosynthesis of plants and cyanobacteria where water is used as electron donor, two photosystems (PS I, PS II) have to cooperate for the conversion of light into chemical energy. In the PS I reaction center, the active species is a special chlorophyll a_I (P-700) [3] and in PS II, a special chlorophyll a_{II} (P-680) [4,5]. Recently, the protein complexes of PS I as well as of PS II of the cyanobacterium *Synechococcus* sp. were isolated and purified in such a way that the stability and homogeneity achieved for the detergent-solubilized complexes permitted estimation of the size, shape and mass of both systems [6,7]. The material produced is optimally suited for the crystallization of these systems. In continuation of these results we report here on the crystallization of the purified protein complex of PS I. During the course of these experiments, Ford et al. [8] recently reported on the crystallization of PS I from another cyanobacterium, *Phormidium laminosum*. Our preparations and crystallization procedures are, however, very different from those in [8], especially in that crystallization was surprisingly effected already at extremely low concentrations, i.e. at concentrations 100–1000-times lower than those usually necessary for protein crystallization. The obtained crystals are furthermore as fully photoactive as the systems in solution and show an X-ray powder diffraction pattern. In some cases, crystals

Correspondence address: H.T. Witt, Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, 1000 Berlin 12, Germany

Abbreviations: APC, allophycocyanin; asc, ascorbate; β-DM, β-dodecyl-D-maltoside; BV, benzyl viologen; Car, carotenoid; Chl, chlorophyll; PEG, polyethylene glycol; PMS, phenazine methosulfate; PS, photosystem; SB, sulfobetain

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

have been obtained with dimensions of up to 0.3 mm.

2. MATERIALS AND METHODS

Cells and membranes of *Synechococcus* sp. were grown and isolated as described [7,9,10]. PS II was nearly quantitatively extracted with 0.3% SB-12. After centrifugation the pellet was extracted with 1% SB-12. The extract was layered on a 10–40% (w/w) sucrose gradient similar to that described in [6], using a buffer with 0.02 M Mes (pH 6.5) and centrifuged in a Beckman SW 27 rotor for 16 h at 23000 rpm (4°C). The resulting green band, called SG-1, was solubilized with buffer A (0.025 M Mes, 0.02 M $CaCl_2$, 0.01 M $MgCl_2$, pH 6.4), containing 0.05% β-DM, and centrifuged for 30 min at low speed to remove unsolubilized particles. The supernatant was supplied to an ion-exchange column set up with Q-Sepharose (Pharmacia). The column was run at 4°C and equilibrated with buffer A together with 0.03% β-DM for retention of the protein. For elution, an $MgSO_4$ gradient between 0 and 0.3 M $MgSO_4$ was applied. Spectra of the different protein fractions were recorded with a Beckman spectrophotometer. Molecular mass estimation was carried out by HPLC-gel filtration with a Superose 6B column (Pharmacia).

Crystallization was performed by the batch method with all fractions from the PS I peak of the ion-exchange column after addition of PEG 6000 (Merck) as precipitating agent in final concentrations between 4 and 8% (w/w). The tubes were left in the dark at 4°C. Respective absorption spectra and dichroisms, of a single crystal were registered with a Zeiss microspectrophotometer (diameter of measuring beam 1 μm).

Photoactivity of the crystals was measured

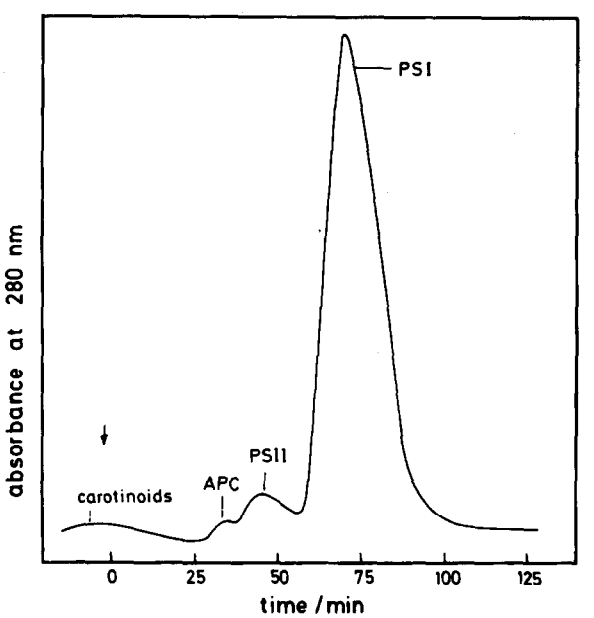


Fig.1. Elution profile of the SG-1 material on a Q-Sepharose column with buffer A (bed volume 6 ml). The start of the $MgSO_4$ gradient (0–300 mM) is indicated by an arrow. Flow rate 1.25 ml/min. Absorption at 280 nm is in the range 0–0.5.

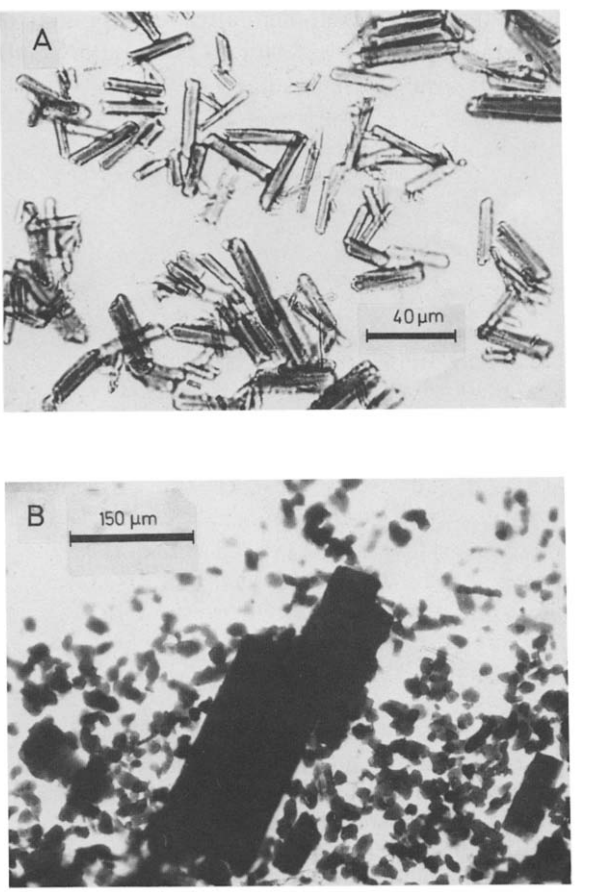


Fig.2. (A) Prismatic crystals after 20 h growth at pH 6.5, 4°C, 5% PEG (w/w) in buffer A; ~25 μM Chl and ~100 mM $MgSO_4$ (color of crystals: light green). (B) A large crystal after 10 days with 4% PEG; 35 μM Chl and ~100 mM $MgSO_4$ (color: dark-green to black).

through flash-induced absorption changes [11].

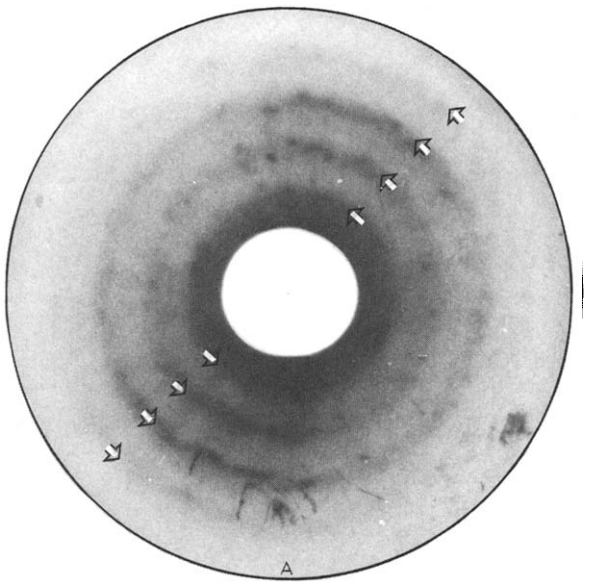
For a first check, 10 μl of a crystal suspension was placed in an X-ray quartz capillary (0.5 mm diameter), centrifuged, and the supernatant removed. The capillary was mounted in a flat film camera with 0.3 mm pinhole collimation and a 200 mm crystal-to-film distance, installed on an Elliott GX6 rotating-anode X-ray generator, operating at 40 kV, 40 mA, and focal spot site 2 × 0.2 mm^2.

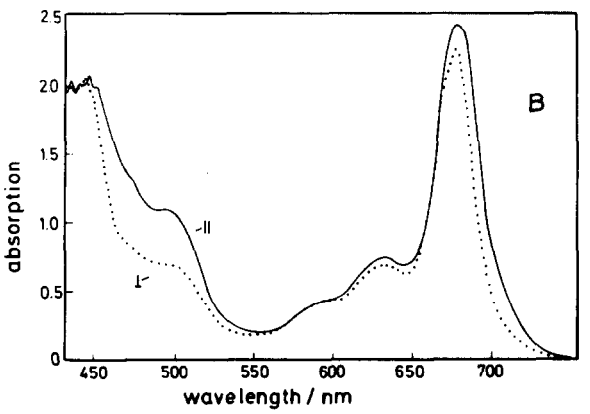
3. RESULTS AND DISCUSSION

Fig.1 shows the elution profile of the SG-1 fraction on the ion-exchange column. First, a carotenoid fraction appears after commencing elution with buffer A and then, after the beginning of the $MgSO_4$ gradient, small peaks of APC and residual PS II are eluted, followed by a large peak of PS I. The species of the fractions were identified by their absorption spectra (not shown). The $MgSO_4$ concentration in the PS I peak was about 100 mM. For crystallization, different fractions of the PS I peak (chlorophyll concentration at the maximum of the peak approx. 35 μM) were mixed with solid PEG 6000 between 4 and 8% (w/w). Small light-green and larger dark-green prismatic crystals appear after half a day to several days.

In contrast to the normal methods of protein crystallization, where usually ≥ 10 mg/ml protein is required, the concentrations used here are at least 100-times lower. Instead of amorphous precipitation, direct crystallization even short (hours) after addition of PEG 6000 is observed.

Fig.2 shows the result of the crystallization pro-





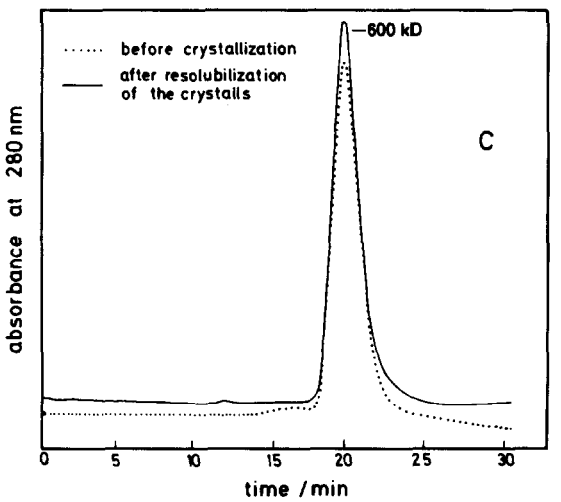


Fig.3. (A) X-ray pattern of crystals (fig.2A). The four rings correspond to spacings of 2.94, 3.63, 4.75 and 6.85 nm between reciprocal lattice planes. (B) Spectrum with polarized light, perpendicular and parallel to the long axis of a single crystal (diameter of measuring beam 1 μm). (C) Elution profile of the PS I fraction on a Superose 6B gel-filtration column (Pharmacia) with buffer A (the PS I fraction was obtained from the ion-exchange column, see fig.1). Profiles before crystallization (···) and after resolubilization of the crystals with buffer A plus 0.09% β-DM (——) are shown. Flow rate 0.7 ml/min.

cedures. Large amounts of uniform, prismatic crystals of about 3–6 μm thickness and 20–40 μm length can be seen (fig.2A). In a few cases, crystals of size up to 300 μm have been registered (fig.2B).

Fig.3A shows an X-ray powder-diffraction pattern of the crystals depicted in fig.2A. The four rings correspond to spacings of 2.94, 3.63, 4.75 and 6.85 nm between reciprocal lattice planes, indicating 'building blocks' of high molecular mass. This, in addition, clearly suggests that the crystals are protein crystals and not crystals of the buffer system covered with absorbed chlorophylls.

Fig.3B shows the spectrum of a single crystal. From the observed absorption of ~2.4 at 680 nm, the crystal thickness and an extinction coefficient of 74000 $M^{-1} \cdot cm^{-1}$ for chlorophyll *a* at 680 nm, one can estimate that the chlorophyll concentration of the crystals is of the order of 0.1 M. This value is about 10^4-times higher than the concentration of the mother liquor. The dichroism of the crystal is recognizable by the different spectra in the polarized light and will be discussed in a forthcoming paper. Similar spectra were obtained by Ford et al. [8] with their crystals.

Fig.3C shows the elution profile on a gel-filtration column of the PS I-protein complex before crystallization and after resolubilization of the crystals. The molecular mass of the protein complex before crystallization has been estimated to be 600 kDa and evidence was given for a trimeric structure of the complex [6]. Since the position of the profile after resolubilization of the crystals is exactly the same as that before, the building blocks within the crystals are probably of the same feature.

In fig.4 the photoactivity of the crystals has been checked. The activity was monitored by observation of the flash-induced absorption changes of the redox reaction of chlorophyll a_I [11]. Crystals placed between a slide and cover slip, respectively, were flashed in the presence of (A) added electron donors and acceptors and (B) in the absence of both. The observed signal shown in fig.4A indicates the photooxidation of Chl a_I and its re-reduction within the crystals. No response is observed without electron donors or acceptors (fig.4B). After the crystals are resolubilized, the signal of the photoreaction of Chl a_I is of the same order of magnitude (not shown) as in the crystals.

We hope that by optimizing in particular the anomal low-concentration method outlined here we shall obtain crystals of a quality suitable for determination of the structure of the PS I complex.

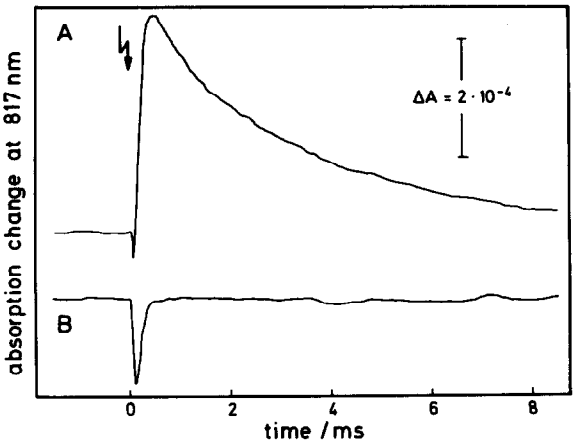


Fig.4. Flash-induced absorption changes, ΔA, of chlorophyll a_I (P700) of crystals in buffer layered on a slide. (A) In the presence of 1 mM PMS with ascorbate in excess as donors and 1 mM BV as acceptor; (B) without acceptors and donors.

ACKNOWLEDGEMENTS

We thank Ms D. DiFiore, Ms I. Geisenheimer and Mrs R. Lindner for excellent technical assistance. We also wish to thank Professor Baumgärtel for kindly providing the Zeiss microphotometer as well as Dr Störmer who helped us with the microphotometer measurements. Financial support from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 312) is gratefully acknowledged.

REFERENCES

[1] Michel, H. (1982) J. Mol. Biol. 158, 567–572.
[2] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[3] Kok, B. and Hoch, B. (1969) in: Light and Life (McElroy, W.D. and Glass, B. eds) pp.397–416, John Hopkins, Baltimore.
[4] Döring, G., Stiehl, H.H. and Witt, H.T. (1967) Z. Naturforsch. 22b, 639–644.
[5] Döring, G., Renger, G., Vater, J. and Witt, H.T. (1969) Z. Naturforsch. 24b, 1139–1143.

[6] Boekema, E.J., Dekker, J.P., Van Heel, M.G., Rögner, M., Saenger, W., Witt, I. and Witt, H.T. (1987) FEBS Lett. 217, 283–286.
[7] Rögner, M., Dekker, J.P., Boekema, E.J. and Witt, H.T. (1987) FEBS Lett. 219, 207–211.
[8] Ford, R.C., Picot, D. and Garavito, R.M. (1987) EMBO J. 6, 1581–1586.
[9] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–14.
[10] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 77–79.
[11] Rumberg, B. and Witt, H.T. (1964) Z. Naturforsch. 19b, 693–707.