Crystallization of the photosynthetic light-harvesting pigment-protein complex B800–850 of *Rhodopseudomonas capsulata*

Wolfram Welte, Thomas Wacker, Monika Leis, Werner Kreutz, Judith Shiozawa*, Nasser Gad'on+ and Gerhart Drews+

*Institut für Biophysik und Strahlenbiologie der Universität Freiburg, Albertstr. 23, 78 Freiburg, FRG, *Dept. of Biochemistry, University of Massachusetts, Amherst, MA 01003, USA,* and *+Institut für Biologie II und Mikrobiologie der Universität Freiburg, Schaenzlestr. 1, 78 Freiburg, FRG*

Received 11 January 1985

The B800–850 light-harvesting complex of *Rhodopseudomonas capsulata* was crystallized in the presence of detergents. The crystals were obtained by a vapour diffusion technique, using polyethylene glycol as a precipitant. Crystals grew to a size of $0.5 \times 0.5 \times 0.3$ mm within two weeks. Two different crystal forms were obtained; one is supposed to be triclinic, space group P1, the other orthorhombic, space group $C222_1$. Both crystal forms diffract to approximately 1.0 nm. Absorption spectra and polyacrylamide gel electrophoresis demonstrate that all expected components, i.e. three polypeptides of apparent M_r 8000, 10000 and 14000, bacteriochlorophyll *a* and carotenoids, are present and in native conformation.

Rhodopseudomonas capsulata *B800–850 light-harvesting complex* *Membrane protein crystallization*

1. INTRODUCTION

Crystallography of membrane proteins has been impossible in the past due to the inability of obtaining crystals. Recently however, several reports of crystallizations of membrane proteins have appeared [1–5], one of which has made possible a crystallographic analysis of the reaction center complex of the purple bacterium *Rhodopseudomonas viridis* at 0.3 nm resolution [6]. The crystallization experiment reported here was carried out with the B800-850 light-harvesting complex from the purple bacterium *R. capsulata*. In most purple bacteria two types of antenna-pigment or light-harvesting (LH) complexes were found, both of which are embedded in the chromatophore membrane [7]. The B870 complex is present in a fixed stoichiometric ratio, and in the neighbourhood of the reaction center, the other variable one (B800-850) is synthesized in variable amounts decreasing with increasing light intensity during growth. Under low irradiance most of the incident photons are absorbed by the pigments of the B800-850 complex. The created singlet states migrate by a random walk as excitons through the LH complexes to the reaction center (B800⟶B850⟶B870⟶P870) [8,9]. Within a short period of less than 0.1 ns, the energy is captured in the reaction center creating a charge-separated state or it is emitted by fluorescence emission.

The B800-850 complex consists of three polypeptides, three molecules of bacteriochlorophyll (BChl) *a* and 1–2 molecules of the carotenoid sphaeroidenone [10–12]. The polypeptides α and β, binding the pigments noncovalently, have M_rs of 7322 and 5087, respectively ([13] and M.H. Tadros, personal communication). The third polypeptide does not bind pigments but is necessary for biosynthesis and stability of the complex [14,15]. The amino acid sequences of the pigment-binding polypeptides each contain a stretch of about 20 hydrophobic amino acids which are surmised to

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

form one transmembrane α-helix [16]. A similar structure has been shown for the corresponding polypeptides of the B800-850 complex of *R. sphaeroides* [17] (the third, non-pigment binding polypeptide is missing) and was confirmed by spectroscopic studies [18–21]. The two B850 BChl molecules presumably form an exciton-coupled dimer giving a strong circular dichroism signal [21]. The B800-850 complex is believed to exist as an oligomer of its basic subunits [12,22]. The apparent M_r was determined to be 180000 [12]. By spectroscopic measurements [23–25] orientations of the transition moments of the pigments in the B800-850 complex of *R. sphaeroides* have been determined. Biochemical and spectroscopic data have been used to propose a model of the B800-850 complex [26]. While this model has to be refined by a high resolution structure analysis, its gross properties show that these membranous LH complexes have a different structure to the water soluble bacteriochlorophyll *a* containing complex of *Prosthecochloris aestuarii* whose structure has been elucidated by X-ray crystallography [27]. Spectroscopic evidences [20] indicate that the B800-850 complex may have some features in common with the LH complex isolated from pea chloroplasts [28]. This complex can be crystallized in two dimensions [29–31] and has been analysed recently by electron microscopic image analysis and three-dimensional reconstruction to a resolution of 15 Å [32].

2. MATERIALS AND METHODS

Lauryldimethylamine oxide (LDAO) was bought from Onyx Chemical Co. (NJ, USA) (Ammonyx LO) and contained 0.7 mM hydrogen peroxide as determined by the Gujakol method [33]. The detergent solution was therefore treated with manganese IV oxide prior to use [34], to decompose the peroxide. Crystalline LDAO was bought from Fluka (Neu-Ulm, FRG). Hexyldimethylamine oxide was synthesized according to [35]. Heptantriol was bought from Oxyl GmbH (Bobingen, FRG). Polyethylene glycol (PEG) 4000 'for gas chromatography' was bought from Merck (Darmstadt, FRG). Standard buffer, called 'buffer' in the following text, was 25 mM Tris-HCl (pH 8.0) at room temperature, plus 5 mM sodium azide. Histo plates, 72 well, sterile, were bought from Dynatech Laboratories (Alexandria, VA, USA). Control spectra of solubilized complexes were routinely measured with a Cary-14 spectrophotometer in the IR1 mode. Spectra of the crystals were measured with a Zeiss microscope, illuminated with a Xenon lamp through a Zeiss PMQII monochromator. Intensity was measured with a GaAs photomultiplier in the spectral range between 410 and 910 nm. The image of the limiting aperture on the crystal had a diameter of 10 μm. A reference intensity was taken closely adjacent to the crystal. The intensity measured through the crystal and the reference intensity were ratiocinated in an HP computer. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [36], with a linear 11.5–16.5% polyacrylamide gradient gel. The B800-850 complex was isolated according to [10], using Onyx LDAO.

Prior to crystallization, Fluka-LDAO was substituted for Onyx-LDAO by binding the protein to a DE52-cellulose (Whatman) column, which was equilibrated with buffer plus 0.08% Fluka LDAO, washed with the same buffer and eluted with a linear sodium chloride gradient in buffer. The main band eluting between 140 and 200 mM sodium chloride was collected. All material eluting later, was discarded.

The protein was concentrated in an Amicon (Amicon GmbH, 5810 Witten, FRG) ultrafiltration cell, equipped with an XM-100 membrane from the same company, up to a concentration where the absorbance $A_{1\,cm,800\,nm}$ reached 120. This corresponded roughly to 10 mg/ml, as determined by the method of Lowry et al. [37]; 6% (w/v) of heptanetriol and 10% (w/v) of hexyldimethylamine oxide were then added to the protein solution.

Buffer solutions containing 12.5% (w/w) PEG 4000 and 1.3 M NaCl, as well as 15% (w/w) PEG 4000 and 1.56 M NaCl were prepared. Five μl of the latter were mixed with 5 μl of the protein solution in a depression of a histo plate and at least 100 μl of the former are given into the remaining depressions as a reservoir. The plate was sealed with parafilm and kept at 22°C in the dark by wrapping with aluminium foil.

For SDS-PAGE, crystals were washed three times (3000 rpm, 5 min, bench top centrifuge) with a buffer solution containing 20% (w/v) PEG 4000

and 1 M NaCl, as well as 0.08% Fluka LDAO and 6% heptanetriol. The crystals were centrifuged once again, supernatant buffer was discarded and the SDS containing solubilizing buffer was added. The sample was heated to 60°C for 20 min and applied to the gel.

3. RESULTS

After 1 to 2 weeks, large crystals appeared with diagonals about 0.5 mm in length. Two different habits were observed: (i) parallel epipeds, which sometimes grow as thin plates, see fig.1 for an example; (ii) coffins.

Absorption spectra of selected thin crystal plates were measured with the microspectrophotometer. They exhibited the characteristic bands of a native and solubilized complex [10]. The spectrum shown in fig.2 was taken from a crystal, it contains the 850 nm absorption peak, which is supposed to arise from a BChl *a* dimer, the monomer Q_y absorption at 800 nm, the Q_x absorption at 590 nm and the carotenoids in the 450–550 nm region.

SDS-PAGE showed the three polypeptide bands of apparent M_r 8000, 10000 and 14000, known from the solubilized complex [11,12] (fig.3).

Crystals of form (a) diffract to about 1.5 nm resolution, the lattice was tentatively assigned to be triclinic, space group P1 with unit cell dimensions of $a = 14.0$, $b = 15.5$, $c = 10.4$ nm, $\alpha = 97°$, $\beta = 104°$, $\gamma = 139°$. Crystals of form (b) diffract to about 1.0 nm resolution, the lattice is orthorhombic, space group $C222_1$ with unit cell dimensions of

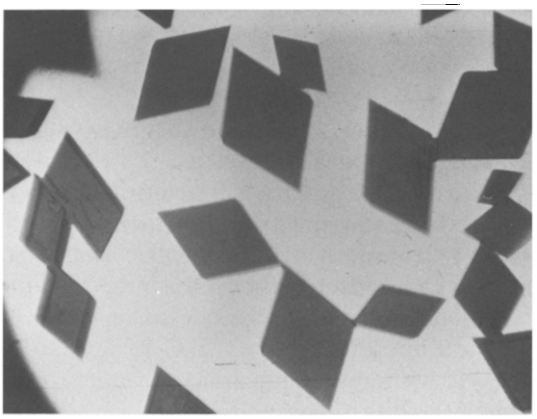

Fig.1. Crystals of the B800-850 LHII complex, triclinic form.

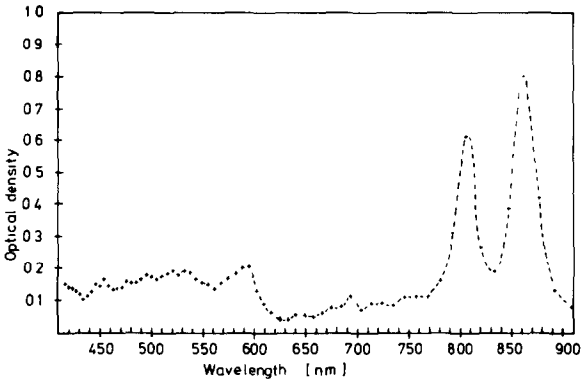

Fig.2. Spectrum of a thin crystal plate of the triclinic type obtained with a microspectrophotometer.

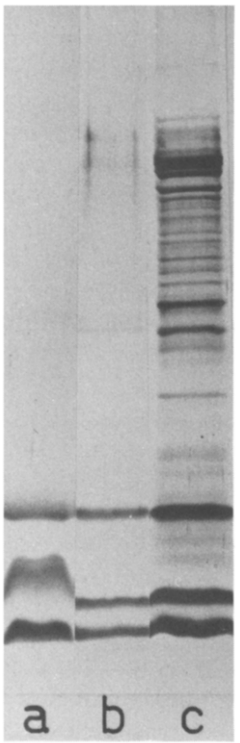

Fig.3. SDS-polyacrylamide gel of LH complex crystals. The crystals were washed three times before application to the gel. (a) Crystals of the B800-850 complex. (b) The isolated B800-850 LH complex. (c) The membrane of the mutant strain Y5 of *Rhodopseudomonas capsulata* from which the B800-850 complex was isolated.

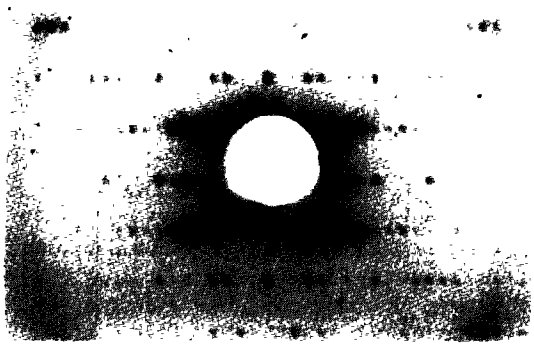

Fig.4. Precession photograph of the 0*kl* plane of the orthorhombic crystals.

a = 15.3, b = 13.3, c = 24.7 nm. Fig.4 shows a precession photograph of the 0*kl* plane.

4. DISCUSSION

All membrane protein crystals mentioned above, probably represent a lattice formed by mixed protein-detergent micelles [5,38,39]. As interactions of micellar surfaces exist in aqueous detergent solutions, they can be expected to play a role in crystal formation. Phase diagrams of several detergents above the critical micelle concentration exhibit temperature-dependent phase separations into a detergent-rich and a detergent-poor phase [40,41]. On approaching the phase separation line, the micelles aggregate to form clusters and ultimately a continuous phase of aggregated micelles [41]. Addition of polyethylene glycol or ammonium sulfate eventually shifts the separation line to room temperature. Possibly these substances effect micellar aggregation by the same mechanisms as protein aggregation. Depending on the type of membrane protein, two limiting forms of the resulting mixed protein-detergent micelle can be thought of: a micelle with the major part of the surface formed by the polar head groups of detergents, and one where the major part of the surface is formed by hydrophilic protein surfaces. On crystallization of the latter membrane protein, one would have to try to use the interaction of water-exposed hydrophilic protein surfaces for crystal formation, the detergent should play a subordinate role at the crystallization conditions. Neither should its interactions impede crystal formation, nor should it sterically impede the formation of a crystal lattice. The latter requirement led Michel to formulate his 'small amphiphile concept' [39].

On crystallization of the former membrane protein, one would have no other choice than to use conditions close to, or even within the phase separation region, since interactions of the protein hydrophilic surfaces are comparable in strength to the micellar interactions. These crystals would be influenced by the arrangement of the detergent around the protein and by their chemical nature, thus affecting the possible protein interactions. Crystal habits would change upon use of other detergent mixtures.

With respect to the published crystals of membrane proteins, the reaction center of *R. viridis* seems to be a good representative of the second case, while matrix porin and lamB protein correspond more to the first case.

We have improved considerably the stability of the LH protein crystals in the process of testing numerous detergent mixtures. The first crystals frequently transformed to small droplets (phase separations) when the conditions were disturbed, e.g., on removal of the crystals from the histo plates. The tests with detergent mixtures, which will be reported elsewhere, confirmed the small amphiphile concept of Michel and improved stability. The crystals obtained now can be washed, stored for weeks, X-rayed for at least a week without indications of deterioration. We think that this is due to a shift away of the phase separation boundary from the crystallization conditions. Considering these results, the LH complex micelle seems to be an intermediate case between the two extremes, although related more to the reaction center of *R. viridis*.

The produced crystals seem to be useful for spectroscopic work. Microspectrophotometry in polarized light is in progress. For X-ray crystallography, the internal order is still insufficient. We have to determine whether small contaminations or the organization of the complex are responsible for that.

ACKNOWLEDGEMENTS

We gratefully acknowledge much invaluable help from Dr Mike Garavito, who helped us to

familiarize ourselves with membrane protein crystallization. We are also grateful to Dr G.E. Schulz for his support and help with the X-ray work, and to Dr Werner Maentele, Dietmar Hueglin and Professor H. Zimmermann for placing the microspectrophotometer at our disposal.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 60, Teilprojekt D12, Dr 29/30-1).

REFERENCES

[1] Michel, H. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 1283–1285.
[2] Garavito, R.M. and Rosenbusch, J.P. (1980) J. Cell Biol. 86, 327–329.
[3] Michel, H. (1982) J. Mol. Biol. 158, 567–572.
[4] Allen, J.P. and Feher, G. (1984) Proc. Natl. Acad. Sci. USA 81, 4795–4799.
[5] Garavito, R.M., Hinz, U. and Neuhaus, J.M. (1984) J. Biol. Chem. 259, 4254–4257.
[6] Deisenhofer, J., Epp, O., Miki, K., Huber, H. and Michel, H. (1985) J. Mol. Biol., in press.
[7] Cogdell, R.J. and Thornber, J.P. (1980) FEBS Lett. 122, 1–8.
[8] Pearlstein, R.M. (1982) Photochem. Photobiol. 35, 835–844.
[9] Borisov, A.Y. (1978) in: The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R. eds) pp.323–332, Plenum, New York.
[10] Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499–513.
[11] Shiozawa, J., Cuendet, P.A., Drews, G. and Zuber, H. (1980) Eur. J. Biochem. 111, 455–460
[12] Shiozawa, J., Welte, W., Hodapp, N. and Drews, G. (1982) Arch. Biochem. Biophys. 213, 473–485.
[13] Tadros, M.H., Suter, F., Drews, G. and Zuber, H. (1983) Eur. J. Biochem. 129, 533–536.
[14] Feick, R. and Drews, G. (1979) Z. Naturforsch. 34C, 196–199.
[15] Kaufmann, N., Huedig, H. and Drews, G. (1985) Mol. Gen. Genet., in press.
[16] Drews, G. (1985) Microbiol. Rev., in press.
[17] Theiler, R., Suter, F., Wiemken, V. and Zuber, H. (1984) Hoppe Seyler's Z. Physiol. Chem. 365, 703–719.
[18] Theiler, R. and Zuber, H. (1984) Hoppe Seyler's Z. Physiol. Chem. 365, 721–729.
[19] Nabedryk, E., Ambinintsoa, S.A. and Breton, J. (1984) Biochim. Biophys. Acta 765, 380–387.
[20] Breton, J. and Nabedryk, E. (1984) FEBS Lett. 176, 355–359.
[21] Bolt, J.D., Sauer, K., Shiozawa, J.A. and Drews, G. (1981) Biochim. Biophys. Acta 635, 535–541.
[22] Peters, J., Takemoto, J. and Drews, G. (1983) Biochemistry 22, 5660–5667.
[23] Bolt, J. and Sauer, K. (1979) Biochim. Biophys. Acta 546, 54–63.
[24] Breton, J., Vermeglio, A., Garrigos, M. and Paillotin, G. (1981) in: Photosynthesis III (Akoyunoglou, G. ed.) pp.445–459, Balaban Int. Sci. Serv., Philadelphia.
[25] Van Grondelle, R., Kramer, H. and Rijgersberg, C.P. (1982) Biochim. Biophys. Acta 682, 208–215.
[26] Kramer, H.J.M., Van Grondelle, R., Hunter, C.N., Westerhuis, W.H.J. and Amesz, J. (1984) Biochim. Biophys. Acta 765, 156–165.
[27] Fenna, R.E. and Matthews, B.W. (1975) Nature 258, 573–577.
[28] Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263.
[29] Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117.
[30] McDonnel, A. and Staehelin, L.A. (1980) J. Cell Biol. 84, 40–56.
[31] Li, J. and Hollingshead, C. (1982) Biophys. J. 37, 363–370.
[32] Kuehlbrandt, W. (1984) Nature 307, 478–480.
[33] Puetter, J. (1971) in: Methoden der Enzymatischen Analyse (Bergmeyer, ed.) pp.725–731, 2nd edn, Verlag Chemie, Weinheim, FRG.
[34] Benjamin, L. (1966) J. Phys. Chem. 67, 3790.
[35] Desnoyers, J.E., Roberts, D., DeLisi, R. and Perron, G. (1982) in: Solution Behavior of Surfactants (Mittal, K.L. and Fendlar, E.J. eds) vol.I, pp.343–358, Plenum, New York.
[36] Laemmli, U.K. (1970) Nature 227, 680–685.
[37] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[38] Garavito, R.M., Jenkins, J., Jansonius, J.N., Karlsson, R. and Rosenbusch, J.P. (1983) J. Mol. Biol. 164, 313–327.
[39] Michel, H. (1983) Trends Biochem. Sci. 8, 56–59.
[40] Balmbra, R.R., Clunie, J.S., Corkill, J.M. and Goodman, J.F. (1962) Trans. Faraday Soc. 58, 1661–1667.
[41] Zulauf, M. and Rosenbusch, J.P. (1983) J. Phys. Chem. 87, 856–862.