Crystallization and spectroscopic investigation with polarized light of the reaction center-B875 light-harvesting complex of *Rhodopseudomonas palustris*

Thomas Wacker, Nasser Gad'on+, Andreas Becker, Werner Mäntele, Werner Kreutz, Gerhart Drews+ and Wolfram Welte

Institut für Biophysik und Strahlenbiologie, Albertstr. 23 and +*Institut für Biologie II, Mikrobiologie, Schänzlestr. 1, 78 Freiburg, FRG*

Received 23 December 1985

A pigment-protein complex containing a reaction center and the B875 light-harvesting complex from *Rhodopseudomonas palustris* was purified and crystallized in the presence of detergent. Thin, rectangular crystals were obtained and used for optical spectroscopy. The protein pattern as well as the spectrum of the solubilized and crystallized complex show that the purified complex was crystallized without a major change in its composition and conformation. The absorption band intensities in the plane of the crystal as well as the spectra of the tilted crystal indicate that the 590 nm Q_x transitions are aligned along the long axis of the crystal, while the 875 nm Q_y transitions are aligned along the short axis of the crystal plane. The results favor a model where the antenna bacteriochlorophylls associated with one reaction center have a common Q_x direction and perpendicular to it a distribution of 875 nm Q_y transitions exhibiting a maximum along one direction.

(Rhodopseudomonas palustris) *Reaction center-B875 complex* *Membrane protein crystallization* *Linear dichroism*

1. INTRODUCTION

In the photosynthetic membrane of purple non-sulfur bacteria the reaction center (RC) is in close contact with the light-harvesting complex B875 (B870, B880 or B1020 in other species). This antenna system, together with a second one termed B800–850, absorbs light energy which ultimately is transferred to the primary donor by radiation-less transfer (review [1]).

By use of suitable detergents and chromatographic procedures, the RC [2–4], the RC–B875 complex [5–10] and the B800–850 complex [8–11] have been purified. The crystallization of integral membrane proteins was recently reported [2,12–15]. The high-resolution X-ray analysis of the photochemical RC of *Rhodopseudomonas viridis* [16] gave important results. The crystallization of the B800–850 complex of *Rps. capsulata* was recently reported by us [17]. In addition, we have also obtained crystals of the corresponding complex of *Rps. palustris* (unpublished). In this article the crystallization of the RC-B875 complex of *Rps. palustris* will be reported.

The smallest unit of the B875 antenna complex consists of two polypeptides of about 6 kDa with a 1:1 stoichiometry [18,19], two bacteriochlorophyll (BChl) molecules and 1–2 carotenoids [20]. In the photosynthetic membrane of *Rps. viridis*, the RC-B1020 complex is the predominant protein component [21] and forms a hexagonal lattice [22,23]. The electron microscopic image of this complex suggests that it might consist of a central RC and peripheral B1020 particles [24,25]. The image of these particles can be enhanced by electron microscopic image filtration [21,24,26–28]. Amino acid sequences, protease digestion studies, IR- and UV-CD measurements [29–31] indicate one

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50 © 1986 Federation of European Biochemical Societies

transmembrane α-helix in each of the two antenna polypeptides. Antibody labelling showed that the RC is in the center part of this RC-B1020 complex [32]. This result is also supported by chemical cross-linking studies [33].

About 25 mol B875-BChl per mol RC have been determined [34,35]. In accordance with these results Ueda et al. [10], using radioactive labelling, found 10–12 of the smallest units per RC for *Rhodospirillum rubrum, Rps. sphaeroides* and *Chromatium vinosum.*

Exciton coupling of the B880-BChl molecules was shown by CD indicating a close proximity of the two BChl molecules [36,37]. Fluorescence polarization measurements of B875 complexes of *Rps. sphaeroides,* wild type and *R. rubrum* showed a strong depolarization of excitations within the antennae, which is, however, smaller at the long wavelength end of the B875 absorption band at low temperature [38,39]. These authors therefore suggest that a small portion of the antenna BChls mediate the excitation transfer between the bulk of the antennae to the special-pair BChl of the RC. Interestingly, chemical cross-linking also reveals the close proximity of some of the B875 polypeptides with a polypeptide of the RC [33,40]. These latter results as well as the non-circular cross-section of the RC of *Rps. viridis* [41] and its pseudo C_2 symmetry [16] show that the RC-B875 complex must possess an asymmetric structure in contrast to that concluded from electron microscopic image filtering at low resolution [21,24, 26–28].

The depolarization of excitations in isolated RC-B1020 complexes of *Rps. viridis* [35] as well as spectroscopy with polarized light [42] in *Rps. sphaeroides* R26 also showed that the Q_x transition moments of the B875 bacteriochlorophylls are predominantly perpendicular and the Q_y transitions are predominantly parallel to the membrane surface but not oriented preferentially in one direction. However, in these measurements the complexes were oriented only uniaxially. The advantage of a crystal is that there is no inherent randomization of the transition moments around an axis. In addition, the *Rps. palustris* RC-B875 complexes are arranged in a simple geomtry in optically thin crystals, thus allowing one to investigate the orientational distribution of the Q_y transition moments around the RC.

MATERIALS AND METHODS

2.1. *Culture conditions, purification and crystallization*

Rps. palustris, strain 1e5, was cultivated phototrophically. The RC-B875 complex was isolated as described in [8], except that we used 10 mM sodium phosphate buffer (pH 8) throughout the preparation instead of the Tris buffer, the dilution step before application of the sample to the gradient was omitted, the gradient contained only 0.5% (w/v) lauryldimethylamineoxide (LDAO) and the band isolated from the gradient was dialysed against 10 mM phosphate buffer and 0.05% LDAO.

LDAO was bought from Onyx (Ammonyx LO) and treated as described [17]. For chromatofocusing, LDAO 'purum' from Fluka (Neu-Ulm, FRG) was used. The RC-B875 complex was purified further by binding to DEAE-Sepharose Cl-6B, Pharmacia, equilibrated with 10 mM phosphate buffer and 0.05% LDAO, and eluted on a sodium chloride gradient.

Ultimately the complex was chromatofocused (Pharmacia, Uppsala, Sweden) in the presence of 20% (w/v) glycerol and 0.08% (w/v) LDAO. The purified complex was dialysed against 25 mM Tris-Cl, pH 8, containing 0.04% LDAO (buffer A) and concentrated by ultrafiltration (Amicon, Witten, FRG; XM-50 membrane) to a protein concentration of 10 mg/ml. This corresponds to an optical absorbance $A_{1\text{ cm, }875\text{ nm}}$ of 130.0. 5 μl of this solution were mixed with the same volume of 17% (w/v) polyethylene glycol (PEG) 2000 (Merck, Darmstadt, FRG) and 20 mM $MgCl_2$ in buffer A.

The mixture was smoothed out on a cover slide and equilibrated with a reservoir of 18% (w/v) PEG 2000 and 20 mM $MgCl_2$ in buffer A by vapor-phase equilibration. Crystallization was performed at room temperature in the dark. Thin crystals that were grown on cover slides were covered with a spacer of 10 μm thickness and sealed with a second cover slide, thus forming a microcell 10 μm thick. In this way, the crystals were aligned with their largest surface parallel to the microcell windows. The microcell was mounted in such a way that rotation of the crystal plane (rotation angle, α) and tilting by up to $\pm$ 30° with respect to the beam direction (tilt angle, β) were possible.

2.2. *SDS-polyacrylamide gel electrophoresis (PAGE)*

SDS-PAGE was performed as described [17]. Crystals were washed 3 times with buffer A containing 16% PEG and 20 mM $MgCl_2$. The crystals were then spun down again, dialysed against water and solubilized in SDS-containing buffer.

2.3. *Spectroscopic techniques*

Absorbance and linear dichroism spectra of the crystals were recorded on a home-built single-beam microspectrophotometer. Briefly, light from a tungsten-iodine lamp was passed through a monochromator and a polarizer, and focused onto the crystal using a microscope objective as an illuminating condensor. A second microscope objective was used to project the magnified image of the crystal onto a rectangular aperture used to cut off false light around the crystal. The light transmitted through the crystal was then focused onto a silicon diode detector with a low-noise preamplifier. An absorbance spectrum was obtained by taking a reference spectrum next to the crystal, a sample spectrum through it and forming $\log(I_0/I)$ on a computer.

Using the microcell described above, the polarization vector of the incident light beam can be rotated in the plane of the microcell (rotation series), as well as be tilted up to an angle of $\pm 30°$ to this plane (tilting series). Upon tilting, the path length of the light through the crystal increases. This increase is a function not only of the tilt angle, but also of the refractive indices of the mother liquor and of the crystal. To account for the different path lengths, for each tilt angle the spectra were taken with polarization perpendicular and parallel to the tilt axis. The second one is altered as compared to the corresponding spectrum at 0° by the increased path length. Each pair of spectra at a given tilt angle, with the polarizer perpendicular and parallel to the tilting axis, therefore was multiplied by the factor which transforms the second spectrum to the reference spectrum, taken at tilt angle 0° and with the polarizer parallel to the tilting axis.

3. RESULTS

3.1. *Purification and crystallization*

The purified RC-B875 complex contained 5 polypeptides in SDS-PAGE: the RC bands at apparent M_r 29000, 26000 and 24000, and the B875 light-harvesting bands at apparent M_r 9500 and 8000 (fig.1). The spectrum of the purified complex is shown in fig.3 (dotted curve).

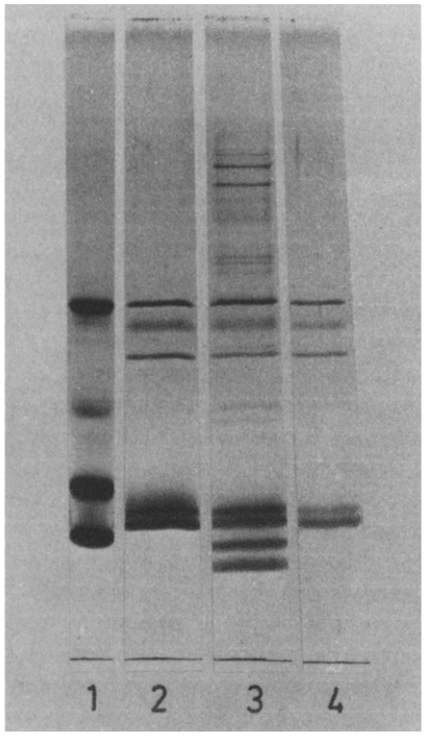


Fig.1. Polypeptide pattern of purified membranes (lane 3), of the purified (lane 4) and of the crystallized (lane 2) RC-B875 complex of *Rps. palustris*. By use of marker proteins, the apparent M_r values of the RC were found to be 29000, 26000 and 24000 and of the B875 complex to be 9500 and 8000. The marker proteins with M_r values of 29000, 21000, 12500 and 6500 are shown in lane 1.

Within one day, the crystallization procedure yields crystals of rectangular form with edge-lengths up to 30×50 μm. SDS-PAGE of washed crystals (fig.1) shows the same polypeptide bands as the purified complex.

3.2. *Spectroscopy*

Fig.2 shows a series of spectra obtained with different angular positions of the polarization vector in the plane of the crystal (rotation series). The rectangular form of the crystal is used to define 3 orthogonal axes, x, y and z, as shown in fig.3c. The 590 nm Q_x band is seen only if the light is polarized

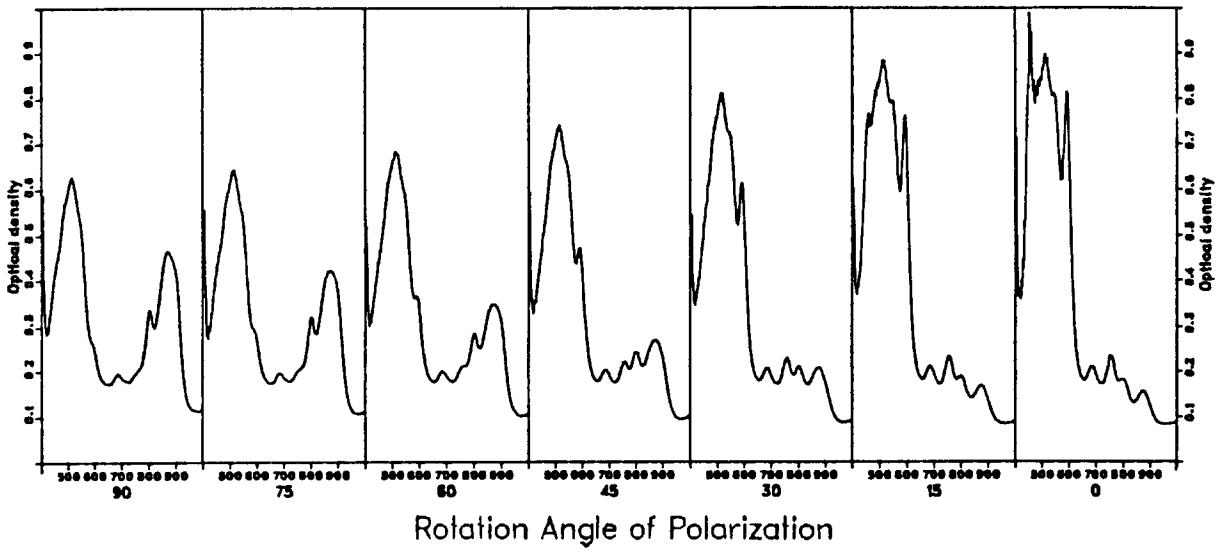
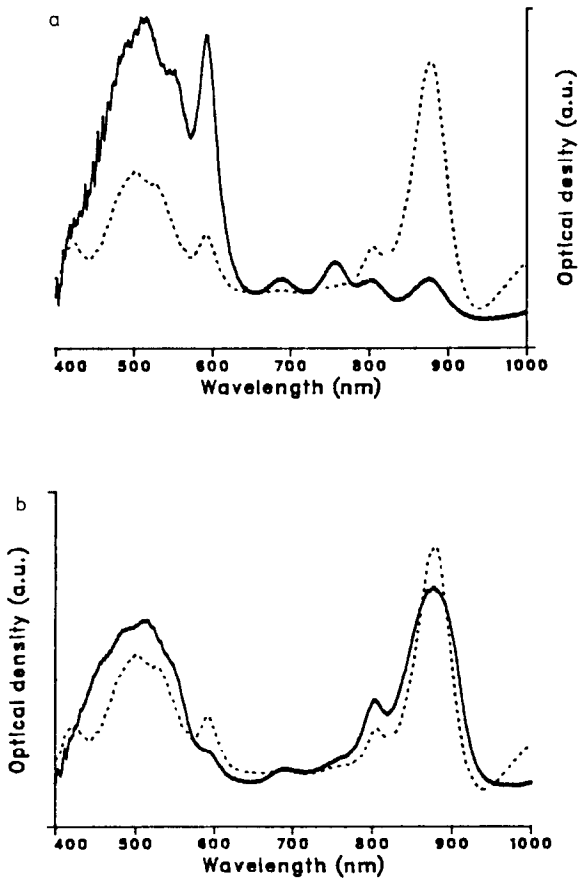


Fig.2. Spectra obtained with different angular positions of the polarization vector in the plane of the crystal (rotation series). The angular position is indicated with respect to the x axis (see fig.3c). The spectra obtained at 0 and 90° are termed I_x and I_y, respectively.



along the x edge of the crystal, while the 875 nm Q_y band is observed only along the y edge. These spectra with polarization along the edges will hereafter be termed I_x and I_y spectra and are shown superimposed in fig.3a and b on the spectrum of the purified complex before crystallization. The almost complete absence of a band at 680 nm in the I_x and I_y spectra clearly indicates that upon

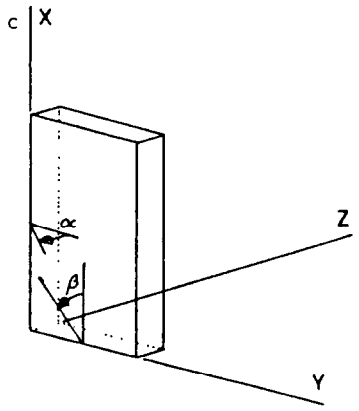


Fig.3. The spectrum of the solubilized complex (dotted curve) is shown superimposed with I_x (a, solid line) and I_y (b, solid line). (c) The diagram defines axes of a coordinate system used to designate the edges of the crystal in the untilted position.

crystallization no oxidation of the pigments occurs. By comparison of the solution spectrum and the I_y and I_x spectra (fig.3a,b) it can be seen that the peak positions coincide closely. The half-width of the Q_y bands in the I_x and I_y spectra, however, seems to have increased.

If the integrals of the Q_x and Q_y absorption bands are formed numerically or by using a planimeter, the ratio of these bands is found to be Q_y/Q_x (solution) = 7–8. If the same ratio is formed from Q_x of the I_x spectrum and Q_y of the I_y spectrum, a much smaller value of Q_y/Q_x (crystal plane) is found. This means that a greater percentage of BChls contributes to the I_x spectrum than to the I_y spectrum. This rotation series as well as the tilting series along the *y* axis (fig.5) show the almost perfect alignment of the Q_x transitions along the *x* edge of the crystal. The Q_y transitions therefore must be distributed in the *y*-*z* plane of the crystal.

More evidence is provided by tilting the crystals. The tilting series along the *x* axis (fig.4) shows the decrease of the 875 nm band with increasing tilt angle (the spectrum with tilt angle 0° corresponds to the I_y spectrum in fig.2 with rotation angle 0°). The tilting series along the *y* axis (fig.5) shows the rise of the 875 nm Q_y transition band at higher tilting angles (the spectrum with tilt angle 0° corresponds to the spectrum I_x in fig.2 with rotation angle 90°). Both series indicate that the Q_y transitions have a preferential orientation along the *y* edge, but also components in the *z* direction. The tilting series therefore indicates a dichroic distribution of the Q_y transitions in the *y*-*z* plane. Non-dichroic distributions of Q_y moments of B875 in the *y*-*z* plane can be excluded. Various planar arrangements of Q_y transitions would result in a vanishing dichroism: examples are randomly distributed Q_y moments, as well as 'stars' formed by the action of C_3 and higher symmetry axes on a single transition moment.

In addition to the large dichroism of the B875 Q_x and Q_y transitions, strong dichroism is also observed in the rotation series (fig.2) for the Q_y transition of the bacteriopheophytin (BPheo) in the RC at 755 nm. The BPheo Q_y orientation correlates closely with the B875 Q_x transitions, i.e. their projection on the crystal plane is parallel to the *x* edge of the crystal.

4. DISCUSSION

Our results show identical polypeptide composition as well as identical positions of absorption bands in spectra of the purified and the crystallized material. This demonstrates that the whole complex was crystallized from solution without a major change in its conformation. In addition, the crystals also exhibit reversible photooxidation of the RC primary electron donor (unpublished).

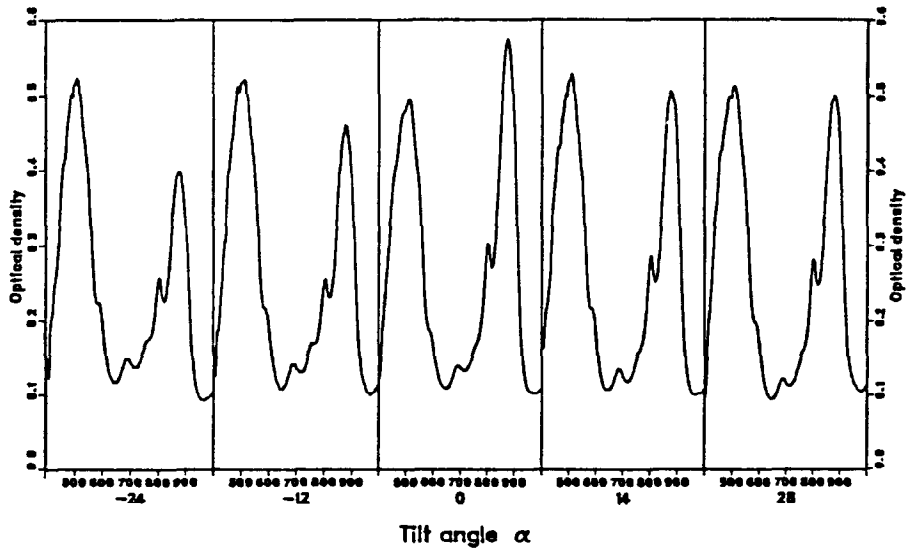


Fig.4. Spectra obtained with the crystal tilted round the *x* axis and the polarization vector parallel to the *y* axis. The angular position is indicated relative to a 0° orientation shown in fig.3c.

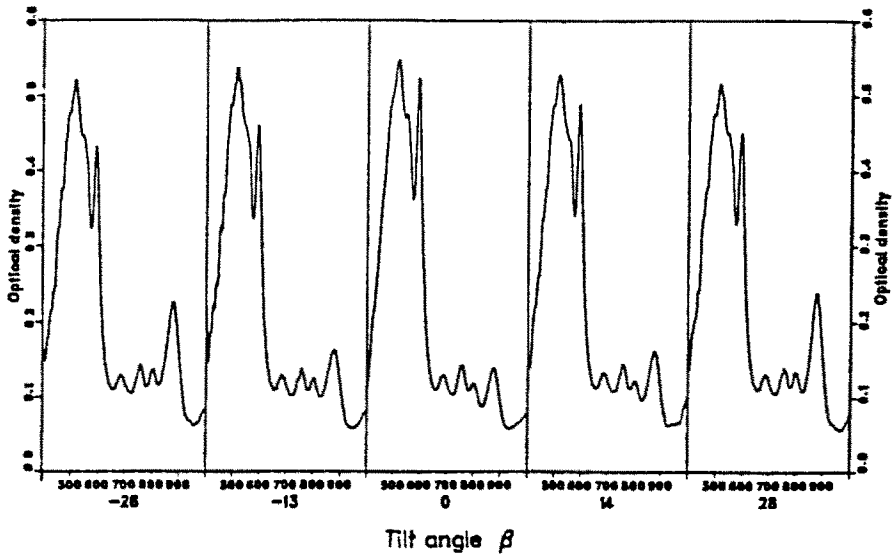


Fig.5. Spectra obtained with the crystal tilted round the *y* axis and the polarization vector parallel to the *x* axis. The angular position is indicated relative to a 0° orientation shown in fig.3c.

Assuming that the RC-B875 complex of *Rps. palustris* is of similar size to the one in *Rps. sphaeroides, R. rubrum* and *C. vinosum* [10], the molecular mass of the complex should be approx. 280 kDa. It is thus larger than the other complexes crystallized previously. In addition to the thin crystalline platelets reported here, we have also obtained large, bipyramidal crystals of the complex in other experiments.

As each symmetry operation of a crystal may diminish the dichroism, it can be deduced from the spectra that the RC-B875 complexes must be arranged in a simple geometric manner in the crystal. A probable arrangement could be that the pseudo C_2 axes of the RCs [16] are all parallel or mutually antiparallel. In addition, they cannot possess a 3-, 4- or 6-fold symmetry axis along this direction.

Although it cannot a priori be excluded that the B875 complexes have rearranged during the process of crystallization, our dichroism spectra demonstrate that the B875 BChls are ordered in a well defined manner around the RC. As in membranes [42] the Q_x transition moments are predominantly aligned along one axis, in our case the *x* edge of the crystal. Therefore the Q_y transition moments have to be in the *y*-*z* plane. The conclusions from our dichroism spectra will be compared to models of the RC-B875 complex, assuming that: (i) The isolation procedure reported here yields the complete RC-B875 complex of *Rps. palustris*. (ii) No rearrangement of the B875 complexes has taken place during the crystallization process.

The models which were based upon the observed fluorescence depolarization in *Rps. viridis* [35] cannot hold for the *Rps. palustris* complex. In those models, the Q_y transition moments are distributed non-dichroic within their plane, i.e. randomly or orthogonally [35].

Our spectra would be consistent with a preferential orientation of the Q_y transitions along a certain direction or with Q_y moments which are pairwise arranged at $\pm\,\phi$ relative to a common direction, ϕ being smaller than 40°, or with a mixture of mutually orthogonal transitions and preferentially oriented transitions.

The spectra also revealed a predominantly parallel alignment of the B875 Q_x transitions and the Q_y transition of the RC BPheo. This correlation would be expected from the arrangement of the BPheo Q_y transitions in the RC of *Rps. viridis* [16,43] and of the B875 Q_x transition [42], both aligned predominantly perpendicular to the membrane plane.

From the dichroism of the B875 Q_x transitions and the RC BPheo Q_y transitions, only the pseudo C_2 orientation relative to the antenna BChls can be determined. At present, we cannot determine the angular position of the RC relative to the *x*-*y* crystal plane, which represents the preferential orientation of the B875 BChl planes. We hope that

future work will help to find the orientation of the RC BChl transitions relative to the B875 transitions.

ACKNOWLEDGEMENTS

We are grateful to Professor J. Breton for helpful discussions and critical reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Drews, G. (1985) Microbiol. Rev. 49, 59-70.
[2] Michel, H. (1982) J. Mol. Biol. 158, 567-572.
[3] Gingras, G. (1978) in: The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R. eds.) pp. 119-129, Plenum, New York.
[4] Welte, W., Hüdig, H., Wacker, T. and Kreutz, W. (1983) J. Chromatogr. 259, 341-346.
[5] Garcia, A., Thornber, J.P. and Vernon, L.P. (1956) Methods Enzymol. 23, 305-320.
[6] Hayashi, H., Miyao, M. and Morita, S. (1982) J. Biochem. 91, 1017-1027.
[7] Jay, F., Lambillotte, M., Stark, W. and Mühlethaler, K. (1984) EMBO J. 3, 773-776.
[8] Firsow, N.N. and Drews, G. (1977) Arch. Microbiol. 115, 299-306.
[9] Varga, A.R. and Staehelin, L.A. (1985) J. Bacteriol. 161, 921-927.
[10] Ueda, T., Morimoto, Y., Sato, M., Kakuno, T., Yamashita, J. and Horio, T. (1985) J. Biochem. 98, in press.
[11] Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499-513.
[12] Garavito, R.M. and Rosenbusch, J.P. (1980) J. Cell Biol. 86, 327-329.
[13] Michel, H. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 1283-1285.
[14] Allen, J.P. and Feher, G. (1984) Proc. Natl. Acad. Sci. USA 81, 4795-4799.
[15] Garavito, R.M., Hinz, U. and Neuhaus, J.M. (1984) J. Biol. Chem. 259, 4254-4257.
[16] Deisenhofer, J., Epp, O., Miki, K., Huber, H. and Michel, H. (1985) J. Mol. Biol. 180, 385-398.
[17] Welte, W., Wacker, T., Leis, M., Kreutz, W., Shiozawa, J., Gad'on, N. and Drews, G. (1985) FEBS Lett. 182, 260-264.
[18] Tadros, M.H., Frank, G., Zuber, H. and Drews, G. (1985) FEBS Lett. 190, 41-44.
[19] Theiler, R., Suter, F. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 703-719.
[20] Cogdell, R.J., Lindsay, J.G., Valentine, J. and Durant, I. (1982) FEBS Lett. 150, 151-154.
[21] Welte, W. and Kreutz, W. (1982) Biochim. Biophys. Acta 692, 479-488.
[22] Giesbrecht, P. and Drews, G. (1966) Arch. Mikrobiol. 54, 297-330.
[23] Welte, W. and Kreutz, K. (1985) Arch. Microbiol. 141, 325-329.
[24] Wehrli, E. and Kübler, O. (1980) in: Electron Microscopy at Molecular Dimensions (Baumeister, W. and Vogell, W. eds) pp. 48-56, Springer-Verlag, Berlin.
[25] Hodapp, N. and Kreutz, W. (1980) Biophys. Struct. Mech. 7, 65-95.
[26] Miller, K.R. (1979) Proc. Natl. Acad. Sci. USA 76, 6415-6419.
[27] Stark, W., Kühlbrandt, W., Wildhaber, I., Wehrli, E. and Mühlethaler, K. (1984) EMBO J. 3, 777-783.
[28] Engelhardt, H., Baumeister, W. and Saxton, W.O. (1983) Arch. Microbiol. 135, 169-175.
[29] Brunisholz, R.A., Wiemken, V., Suter, F., Bachofen, R. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 689-701.
[30] Theiler, R. and Zuber, H. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 721-729.
[31] Nabedryk, E., Berger, G., Andrianambinintsoa, S. and Breton, J. (1985) Biochim. Biophys. Acta 809, 271-276.
[32] Stark, W., Jay, F.A., Kühlbrandt, W., Wildhaber, I. and Mühlethaler, K. (1984) in: Advances in Photosynthesis Research, vol. 3, pp. 361-364, Nijhoff/Junk, The Hague.
[33] Peters, J., Takemoto, J. and Drews, G. (1983) Biochemistry 22, 5660-5667.
[34] Aagaard, J. and Sistrom, W.R. (1972) Photochem. Photobiol. 15, 209-225.
[35] Breton, J., Farkas, D.L. and Parson, W.W. (1985) Biochim. Biophys. Acta 808, 421-427.
[36] Hayashi, H., Nozawa, T., Hatano, M. and Morita, S. (1982) J. Biochem. 91, 1029-1038.
[37] Bolt, J.D., Sauer, K., Shiozawa, J. and Drews, G. (1981) Biochim. Biophys. Acta 635, 535-541.
[38] Bolt, J.D., Hunter, C.N., Niederman, R.A. and Sauer, K. (1981) Photochem. Photobiol. 34, 653-656.
[39] Kramer, H.J.M., Pennoyer, J.D., Van Grondelle, R., Westerhuijs, W.H.J., Niederman, R.A. and Amesz, J. (1984) Biochim. Biophys. Acta 767, 335-344.
[40] Peters, J., Welte, W. and Drews, G. (1984) FEBS Lett. 171, 267-270.
[41] Miller, K.R. and Jacob, J.S. (1983) J. Cell Biol. 97, 1266-1270.
[42] Breton, J. (1974) Biochem. Biophys. Res. Commun. 59, 1011-1017.
[43] Breton, J. (1985) Biochim. Biophys. Acta 810, 235-245.