Antennas and Reaction Centers of Photosynthetic Bacteria

Structure, Interactions, and Dynamics

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Contents

Single Crystals from Reaction Centers of *Rhodopseudomonas viridis* Studied by Polarized Light

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The spectral properties and the pigment composition of isolated reaction centers (RC) have been studied extensively in the past /1-3/. Recently the reaction centers from Rhodopseudomonas viridis (Rps. viridis) were crystallized /4/ and an X-ray structure analysis yielded the pigment arrangement /5/. From the latter publication we know that the four bacteriochlorophyll b (BC) and **the two bacteriopheophytine b (BP) molecules are arranged in two branches (see Fig. 1). They are related pairwise by a twofold local symmetry axis (broken line). The pigments of the branch containing the quinone, probably a menaquinone, MQ, are closer to the L protein subunit, the other pigments are closer to the M subunit. Consequently we index them by L and M. It was assumed /5/ that BC_{LP} and BC_{MP} form the special pair (P) and that BC_{LA} and BC_{MA} are the accessory** bacteriochlorophylls.

In this Letter we present the results from optical investigations of the crystallized reaction centers and combine the optical and the structural information. The optical properties of the reaction centers in the single

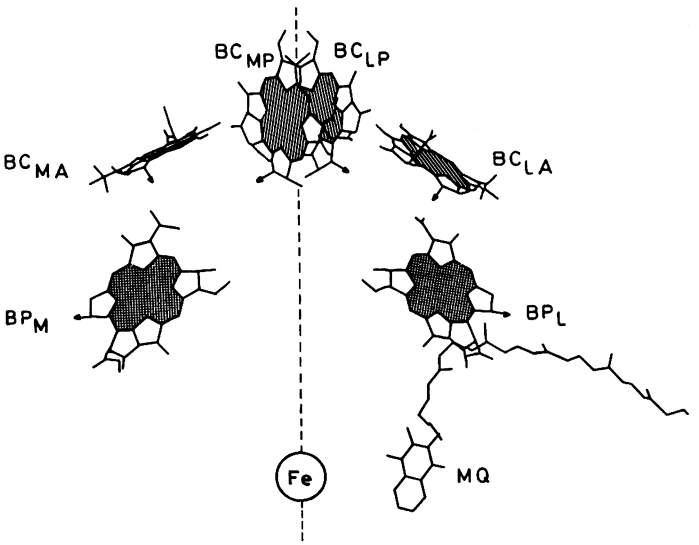

Fig. 1 Model of the central part of the reaction center of Rps. viridis containing the four bacteriochlorophyll b (BC), the two bacteriopheophytine b (BP) molecules and the menaquinone (MQ) after Ref.5. The molecules are arranged in two branches, L and M.

crystals are characterized and the influences of oxidation, reduction and photooxidation on the absorption spectra are shown. We make use of the ordered arrangement of the RC in the crystals to interpret the optical spectra: We assign absorption bands to particular pigments in the molecular model of the reaction center.

The reaction centers were isolated and the crystals were grown as described recently /6,4/. The space group of these crystals is $P4_32_12$. The tetragonal unit cell has the dimension of 223×223×114 A^3 and contains eight asymmetric units. Each asymmetric unit is occupied by one RC molecule yielding a RC-concentration of 2.35×10^{-3} mol/l. In most cases the crystals grow in the form of tetragonal columns with the preferential growth direction parallel to the z-axis. Fortunately, the crystals can be obtained in the form of thin platelets of several hundered micrometers in length and width and with a uniform thickness of a few micrometers. The surfaces of the platelets are parallel to the xy, xz, or yz planes. The orientation of the crystallographic axis can be determined upon microscopic inspection. During the investigations the crystals were kept in closed cells which contained a salt solution of 2.7 M ammonium sulfate, 20 mM sodium phosphate, 0.1% N,N-dimethyldodecylamine N-oxide, 1% triethylammonium phosphate, and 1% heptane-1,2,3-triol, pH 6.5 (chemically reduced crystals were prepared by adding 20 mM sodium ascorbate to the salt solution, whereas oxidation of the RC was accomplished by adding $K_3Fe(CN)_6$ (100 mM).

The transmission spectra were recorded with a self-constructed microscope set-up: Light from a tungsten lamp was monochromized by a first grating spectrometer of 3nm resolution. The light beam of a certain linear polarization was focussed onto the crystal via a microscope objective (illuminated area 70 µm × 70 µm). The intensity of the measuring light in the crystals was kept at the lowest possible level,in order to avoid absorption changes due to photooxidation of the RC. We have confirmed that less than 3% of the RC were photooxidized due to the action of the measuring light. Part of the incoming measuring light was split-off by a beamsplitter and used as a reference. The light transmitted through the crystal was imaged by another objective onto the slit of a second spectrometer, and detected by a suitable photomultiplier (photocathodes S 20 or S 1). The second spectrometer shielded the photomultipliers against stray light. The electrical signals were processed by a phase-sensitive detection system.

Light-induced absorbance changes were measured in the following way: At certain wavelengths the transmitted light power was recorded prior to (I_{dark}) and after illumination ($I_{illum.}$) of the RC by actinic light (for 30 s). The logarithm of the ratio $I_{illum.}/I_{dark}$ yielded the absorbance change ΔA. Actinic light was obtained from a Xe-arc-lamp used in conjunction with a spectrometer transmitting radiation between 960 nm and 980 nm. The actinic light was linearly polarized and travelled in the crystals collinearly to the measuring beam. In order to avoid saturation of the absorbance changes, the level of the actinic light was kept so low, that the relative absorbance changes were always smaller than 10%.

Fig. 2 shows absorption spectra of the RC crystals in different surroundings. The same geometry of observation was used when recording the three spectra: the crystals were oriented with the tetragonal axis perpendicular to the propagation direction (k) of the measuring light. The polarisation (vector E) was parallel (broken curves) and perpendicular (solid curves) to the tetragonal axis.

The absorbance spectrum of the native RC crystals (Fig 2a) displays all features known from RC in solutions. The Q_y absorption bands of the BC and

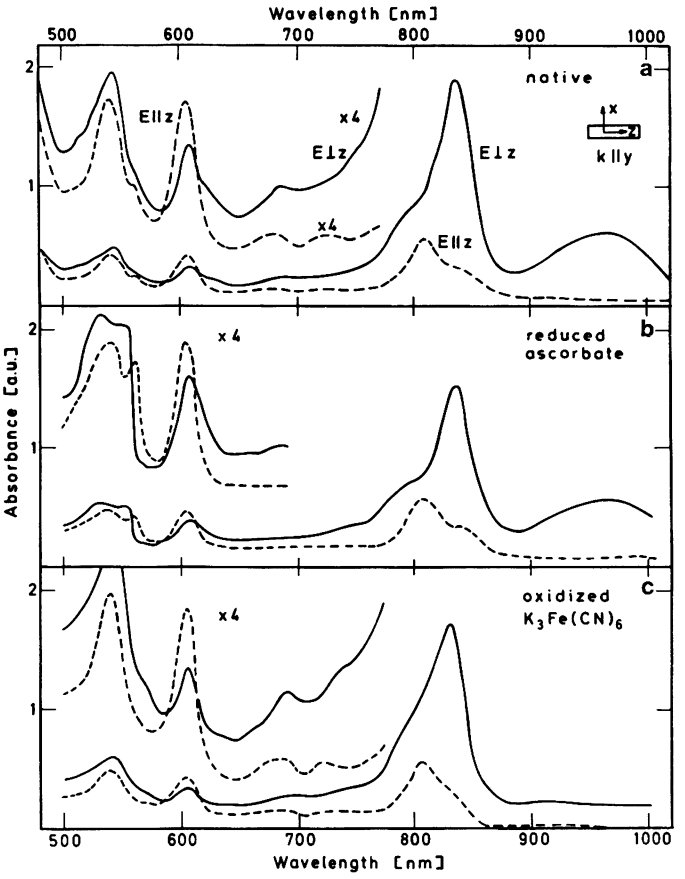

Fig. 2 Polarized absorbance spectra of single crystals of reaction center preparations from Rps. viridis: (a) native crystals, (b) crystals treated with ascorbate, (c) crystals treated with $K_3Fe(CN)_6$.

BP are between 750 nm and 850 nm . Q_y of the special pair (P) is around 960 nm. The Q_x absorption band of the BC is close to 605 nm, whereas the Q_x absorption of the BP and the bands of the cytochrome c are seen near 550 nm. Of special interest is the comparison of the extinction coefficients determined for RC solutions and RC crystals. Using the transmission data for λ = 960 nm, the measured crystal thickness, the known RC concentration and taking into account that the transition moment of the 960 nm band is polarized perpendicular to the tetragonal axis, we obtain the value for the extinction coefficient of ε = 110,000 M^{-1} cm^{-1}. In order to compare the data from RC crystals and RC solutions , the extinction coefficient ε was calculated for isotropically arranged RC. The value determined for the crystallized RC agrees with the published extinction coefficient of ε = 125,000±25,000 $M^{-1}cm^{-1}$ for RC in solutions /2/. This finding gives a strong indication that the RC crystals contain intact RC where the strength of the special pair transition at 960 nm is not influenced by crystallisation.

The polarized absorption spectra allow us to deduce qualitatively the orientation of the transition moments within the RC crystals. The combination with the structural data allows us to assign absorption bands to particular pigments. Detailed information can be obtained for the BP molecules: It is known from the literature that the BP absorb around 800 nm /1/. In the polarized absorption spectra we find one band at 805 nm polarized parallel to the tetragonal z axis, whereas a strong shoulder is seen in the ($E \perp z$) spectrum at 790 nm. Since BP_L has a considerable absorption for light polarized parallel to the z axis, we can assign the absorption at 805 nm to BP_L and the shoulder at 790 nm to BP_M.

RC crystals reduced by ascorbate show changed absorption spectra (see Fig. 2b): Between 550 nm and 560 nm, in the range of the cytochrome c molecules absorption increases considerably. It is known that the RC of Rps. viridis in total contain four cytochrome c molecules. The X-ray structure analysis shows that the four cytochrome c molecules are positioned in the RC roughly along a line pointing away from the special pair. There are two different types, two cytochrome 558 and two cytochrome 553 molecules named according to the position of their long-wave absorption peak. The observed absorbance increase suggests that in the native crystals the cytochrome c molecules are mainly oxidized, having negligible absorption around 555 nm. In the reduced crystals the additional absorption due to the cytochromes has a pronounced dichroism: A peak is seen in the $E \parallel z$ spectrum at 558 nm whereas the $E \perp z$ spectrum displays a shoulder at 558 nm and a peak at 553 nm. The different polarisation properties of the two absorption bands in combination with the results of the structure analysis indicate that the cytochrome molecule which is closest to the special pair is a cytochrome 558.

In Fig. 2c the absorption spectra of RC crystals oxidized by $K_3Fe(CN)_6$ are shown. The 960 nm transition of the special pair disappears and the 830 nm band is shifted to shorter wavelengths. When inspecting more carefully the absorption spectra, one realizes a weak absorbance decrease around 605 nm. In addition, the broad shoulder around 620 nm in the $E \perp z$ spectrum is reduced (as compared with Fig. 2a). The complete lack of any absorption peak between 550 nm and 560 nm suggests that all the cytochrome molecules are oxidized. Finally there is a very broad absorption background extending throughout the visible in the $E \parallel z$ spectrum. This background may be tentatively assigned to the oxidized special pair P^+ (see below).

The action of oxidation on the crystallized RC is seen more clearly when the absorbance changes induced by photooxydation are studied. The relative polarisation of actinic and measuring light in the crystals allows to obtain additional information. In tetragonal crystals different geometries are needed to deduce the complete spectral information. In principle the spectra of Fig. 3 allow to determine the direction fo the transition moments of the pigments in the crystals /7/. As an example we have calculated from the light-induced absorbance changes the direction of the special pair transition at 960 nm. The transition is polarized mainly in the xy-plane (within $\pm 7°$) and at an angle of $30° \pm 5°$ relative to the x-direction. It is interesting to note that this value agrees within the experimental accuracy with the direction determined by excitonically coupling the Q_y-transitions of the BC molecules forming the special pair (BC_{LP} and BC_{MP}). This finding supports the interpretation that: (i) BC_{LP} and and BC_{MP} form indeed the special pair and that (ii) the interaction between the special pair and other pigments does not influence the direction of the 960 nm transition.

The quantitative analysis of the directions of the transition moments in the Q_y range of the BP and the accessory BC is made difficult by the strong overlap of the absorption bands. Nevertheless, some interesting features are readily determined from the absorption spectra of Fig. 3. Around 850 nm the

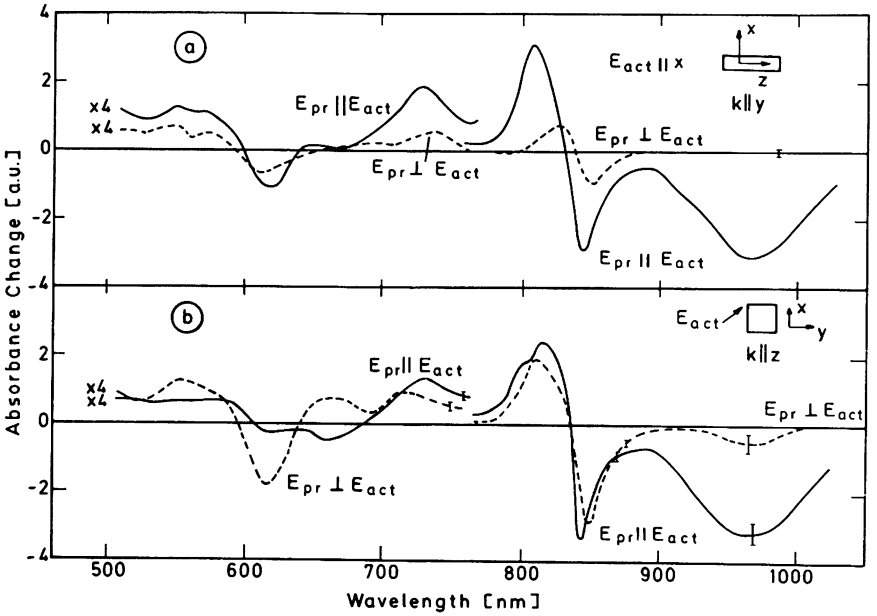

Fig. 3 Absorbance changes of the crystallized reaction centers induced by illumination with actinic light at 970 nm. The propagation direction k of actinic and measuring light is parallel to the y-axis (Fig. 3a) or parallel to the z-axis (Fig. 3b). The electric-field vector of the actinic light (E_{act}) is parallel to the x-axis and at 45° to the x-axis in Fig. 3a and Fig. 3b respectively. The solid and the broken curves were taken with the electric-field vectors of measuring (E_{pr}) and actinic light (E_{act}) parallel and perpendicular to each other, respectively.

difference spectra have a dispersion-type shape. A more careful inspection of the experimental data reveals that the points of zero-crossing, as well as the position of the minima and maxima, do not coincide in the four spectra of Fig. 3. This indicates that the absorption bands show shift and reorientation upon photooxidation. This can be caused by the oxidation of the special pair changing the excitonic interactions and leading to Stark-shifts of the resonance frequencies. In the spectral region of the Q_x bands of the BC one finds a pronounced absorbance decreases at 618 nm with a width of 30 nm. The polarization dependence of that absorbance decrease supports the assignment that the Q_x band of the special pair is at 618 nm. The apparent red-shift of the special pair Q_x transition relative to the absorption band of the accessory BC at 605 nm allows a selective excitation of the special pair by light with $\lambda \approx 620$ nm. This finding is important for the interpretation of very recent time-resolved experiments on the RC /8/.

The detailed optical investigations of the crystallized reaction centers from Rhodopseudomonas viridis give valuable information: (i) The reaction center crystals contain intact reaction centers which are photochemically active. There is quantitative agreement of the absorption cross-section of the special pair in solution and in the crystal. (ii) The reaction centers can be reduced and oxidized in the crystalline form. This allows structural studies from chemically treated reaction centers. (iii) The assignment of absorption bands to particular pigment molecules is achieved.

References

1 J.P. Thornber, P.L. Dutton, J. Fajer, A. Forman, D. Holten, J.M. Olson, W.W. Parson, R.C. Prince, D.M. Tiede and M.W. Windsor in Proceedings of the 4th International Congress on Photosynthesis, 1977 (D.O. Hall, J.Coombs and T.W. Goodwin, eds. The Potochemical Society, London, 1978), pp. 55-70
2 R.K. Clayton and B.J. Clayton: Biochim. Biophys. Acta 501, 478 (1978)
3 J.P. Thornber, R.J. Cogdell, R.E.B. Seftor and G.D. Webster: Biochim. Biophys. Acta 593, 60 (1980)
4 H. Michel: J. Mol. Biol. 158, 567 (1982)
5 J. Deisenhofer, O. Epp, K. Miki, R. Huber and H. Michel: J. Mol. Biol. 180, 385 (1984)
6 W. Zinth, W. Kaiser and H. Michel: Biochim. Biophys. Acta 723, 128 (1983)
7 E.W. Knapp, W. Zinth, M. Sander, S.F. Fischer, W. Kaiser, J. Deisenhofer and H. Michel: PNAS (1985)
8 W. Zinth, M.C. Nuss, M.A. Franz, W. Kaiser and H. Michel, same book.