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EFFICIENT PHOTOCHEMICAL ACTIVITY AND STRONG DICHROISM OF SINGLE CRYSTALS OF REACTION CENTERS FROM *RHODOPSEUDOMONAS VIRIDIS*

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Crystallized reaction centers from *Rhodopseudomonas viridis* (i) are photochemically active with electron transfer from the special pair to the quinones, (ii) show dichroism giving valuable information on the orientation of the different chromophores and (iii) allow chemical treatment in the crystalline phase.

In the past, reaction centers from photosynthetic bacteria have been investigated in intact cells, in photosynthetic membranes or isolated in detergent solutions. Comparison of the optical spectra of intact cells with those of isolated reaction centers suggested that careful isolation does not seriously affect the properties of the reaction centers [1–6]. The spectral properties and the pigment composition of isolated reaction centers from *Rhodopseudomonas viridis* have been studied extensively [1–6] Recently, the photosynthetic reaction centers from this photosynthetic bacterium were crystallized [7]. In this paper we report on the properties of these crystals

Reaction centers isolated with the detergent *N*, *N*-dimethyldodecylamine *N*-oxide contain four BChl *b* and two BPh *b* molecules [1] The primary electron donor, called P-960 due to its maximum of absorbance, is made up of two of the four BChl *b* molecules, while the 'primary' electron acceptor is a quinone-iron complex [1]. There are one menaquinone and one to two ubiquinones per P-960 [8]. Isolated reaction centers from *Rps viridis* also contain four heme groups (two 'cytochrome c-558' and two 'cytochrome *c*-553'), which are probably bound to the same protein subunit [6]. They feed electrons to P-960$^+$.

The reaction centers were isolated as described recently with the following modification [7]. For the molecular sieve chromatography step we used as column material Fractogel TSK HW 55 S (Merck, Darmstadt), this step was repeated. In contrast to earlier preparations [7] using different column material, we now observe bleaching of P-960 upon illumination This finding indicates that the quinones are still present in the new preparations. The crystals were grown as described previously [7]. The space group of these crystals is $P4_{1(3)}2_12$ with a tetragonal unit cell dimension of $223 \times 223 \times 114$ Å containing eight asymmetric units per crystallographic unit cell. The volume of the asymmetric unit and the molecular weight of the reaction centers make it more likely that each asymmetric unit is occupied by two reaction center molecules than by one or three With these figures were calculate $2.8 \cdot 10^{18}$ reaction center molecules per cm^3, corresponding to $4.7 \cdot 10^{-3}$ mol/l. In space group $P4_{1(3)}2_12$, a 4-fold screw axis is parallel to the *z*-direction whereas nonintersecting 2-fold screw axes (perpendicular to the 4-fold axis) are

Abbreviations BChl, bacteriochlorophyll, BPh, bacteriopheophytin

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parallel to the x- and y-direction. 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 hundred micrometers in length and width and with a uniform thickness of a few micrometers whilst the surfaces of the crystals are still parallel to the xy, xz, or yz planes In these platelets the orientation of the crystallographic axes can be determined upon visual inspection

Spectral transmission measurements and observations of the photochemical activity were made with the help of two spectrometers, a microscope, and various photodetectors Care was taken to keep the measuring light at the lowest possible level to avoid absorption changes due to photochemical activity during the spectral transmission measurements. Photoreactions were studied by side illumination of the crystal with actinic light of $\lambda > 850$ nm (incandescent lamp plus cutoff filter). The photodetector was protected against scattered light by one of the spectrometers. During our investigations the crystals were kept in a closed cell, 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.

The crystals are uniform in their absorption properties Scanning the cross-section of the crystals or determining the extinction coefficient for different crystal thicknesses suggests a high degree of crystal homogeneity. It should be emphasized at this point that the same absorption spectrum was obtained from different crystals prepared over a period of several months.

The optical spectrum of the reaction centers in the starting solution is shown in Fig. 1a; it is essentially identical to absorption data found in the literature for *Rps. viridis* in solution [1–5].

The optical absorption spectrum of reaction center crystals from *Rps viridis* is presented in Fig 1a (broken line) and the change in absorption versus wavelength for irradiation with infrared light at $\lambda \geq 850$ nm is shown in Fig. 1b. All spectra are recorded with unpolarized light. The difference spectrum gives positive and negative contributions to the absorption spectrum taken in the 'dark'

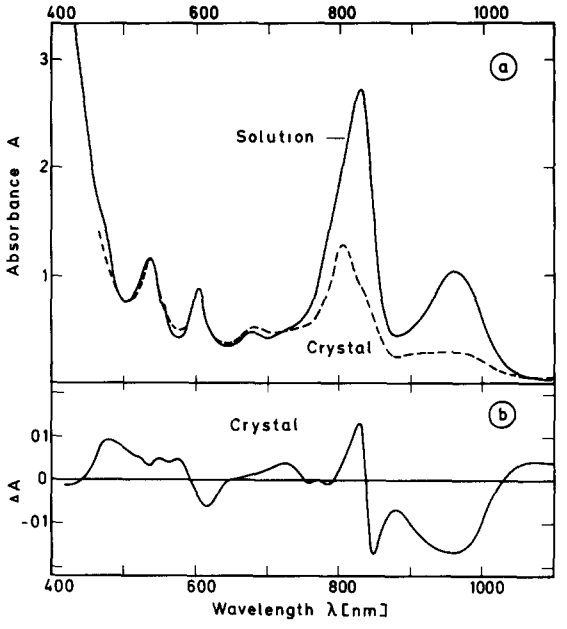

Fig 1 (a) Unpolarized absorption spectra of reaction centers from *Rps viridis* of a solution (solid curve) and of a single crystal (broken line) The crystal was oriented with its z-axis perperdicular to the measuring light beam The reaction center concentration of the solution was adjusted to have equal absorbance at $\lambda = 530$ nm (b) Absorbance change of the crystal upon illumination with actinic light with $\lambda \geq 850$ nm

Qualitatively, the difference spectrum is similar to that in published data on reaction centers in solution, but the dichroism of the crystals (see below) gives rise to quantitative changes (e.g., at 960 and around 830 nm) [3,9,13].

Comparing the absorption spectrum of the reaction centers in solution and in the crystal (Fig. 1a) we note drastic differences of the strong absorption around 830 nm and of the absorption band at 960 nm. It is generally accepted [1] that the strong band at 830 nm is mainly due to two molecules of BChl *b* and that the maximum at P-960 is indicative of the exciton-bound dimer consisting of the other two BChl *b* molecules. The smaller absorption of unpolarized light at 830 nm in the crystal is due to the strong dichroism which will be discussed below The reduced absorption at 960 nm in the crystals has two causes First, it results from the dichroism of our samples and, second, it is caused – to some extent – by the oxidation of special pairs. In fact, we find absorption at 1 3 μm (without excess illumination) in the

crystals suggesting the existence of P-960$^+$ states frozen in at room temperature. Furthermore, we made the interesting observation that chemical treatment of the small crystals is possible. The addition of the reducing agent ascorbate to the liquid surrounding of the crystals leads to an increase in the absorbance at 960 nm by approx 20%, quite likely, P-960$^+$ radicals are reduced At the same time we find an enhanced absorption at 558 nm which points to a reduction of cytochrome *c* present in the reaction center. This chemical treatment does not disorder the crystals as checked by X-ray diffraction.

Of special interest is the finding that the P-960 band in our crystals bleaches completely and reversibly upon illumination with radiation of $\lambda \geq$ 850 nm The photoefficiency appears to be high, we estimate a value between 0.1 and 1. Our data show that photoactivity, i e., the charge separation in the special pair and the charge transfer to an acceptor (probably the quinones), is preserved upon crystallization. In fact, the difference absorbance spectrum of Fig. 1b is similar to published spectra reported to be characteristic of the P^+Q^- states [3,9]

Fig. 1a shows a shoulder around 850 nm and in Fig. 1b we found a distinct bleaching at the same wavelength. This observation agrees with the interpretation that the absorption at 850 nm represents the second dimer band of the special pair [1,9,10]. CD investigations [11], studies of oriented cells [12], and our measurements on crystals with polarized light (see below) lead to the same conclusions

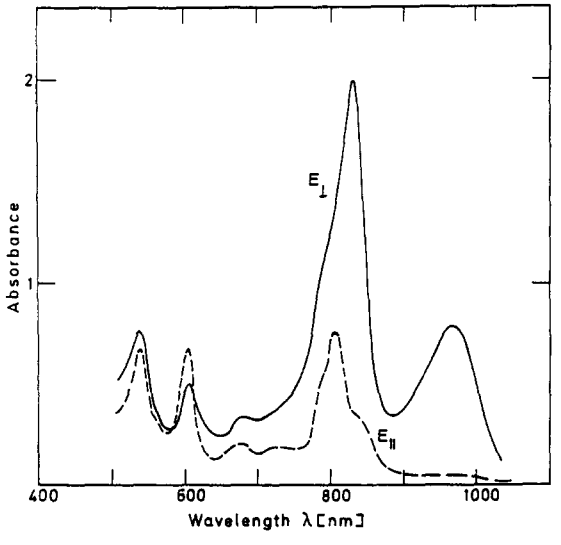

Fig 2 Polarized absorption spectra of a single crystal of reaction center preparations from *Rps viridis* The electric vector of the measuring light was chosen perpendicular (solid curve) and parallel (broken line) to the *z*-axis

The small absorption band at 680 nm is an oxidation product of BChl *b* [2]. Special care in avoiding oxygen during the preparation reduces this minor absorption band.

In Fig 2 we present transmission studies of the crystals, where the light was polarized with the electric vector either parallel or perpendicular to the 4-fold screw axis (*z*-axis) with the beam perpendicular to the surface of the platelets. The two absorption spectra of Fig 2 show drastic differences over the whole spectral range. Most striking is the disappearance of the dimer peak P-960 for the $E_\parallel$ polarisation and the very strong absorption of the BChl *b* molecules at 830 nm for $E_\perp$ The data of Fig. 2, while not sufficient for a final assignment of the orientation of the different chromophores, give a series of new information on pigment orientation. (i) The strong absorption of P-960 for $E_\perp$ suggests that the major dimer axis is located perpendicular to the *z*-axis of the crystals, while the absorption band at 850 nm (assumed to be the second dimer absorption) has components parallel to the *x*-axis as seen from the shoulder in the $E_\perp$ spectrum. (ii) The two BChl *b* molecules, which are thought to absorb at 830 nm at room temperature, are also strongly oriented in the crystals. They have their major Q_y absorption perpendicular to the *z*-axis. The Q_x transition of the same molecules give rise to the enhanced absorption in the $E_\parallel$ spectrum at 600 nm (iii) The two BPh *b* molecules absorb at 790 and 810 nm, according to the literature [9] Our absorption data of the $E_\parallel$ spectrum show a maximum at 805 nm and a shoulder around 780 nm. Furthermore, in the $E_\perp$ spectrum a shoulder appears around 800 nm These data suggest that the BPh *b* molecules have an orientation substantially different from that of the BChl *b* molecules. The Q_y band of the two BPh *b* molecules absorbs around 800 nm in the $E_\parallel$ and $E_\perp$ spectrum and the enhanced absorption at 530 nm in the $E_\perp$ spectrum is due to the Q_x transitions

More detailed studies on the dichroism and the photochemical activity of the crystals are in progress

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Note added in proof (Received March 24th, 1983)

Protein determinations using crystals of known volume suggest that the crystals contain only one reaction center molecule per asymmetric unit

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