Fluorescence-ODMR of Reaction Centers of *Rhodopseudomonas viridis*

A. Angerhofer, J. U. von Schütz, and H. C. Wolf

Physikalisches Institut, Teilinstitut 3, Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80, Bundesrepublik Deutschland

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Reaction Centers of *Rhodopseudomonas viridis*, Bacteriochlorophyll *b*, Fluorescence-ODMR

Fluorescence detected ODMR measurements in zero field on the triplet state of the reaction center in whole cells of *Rhodopseudomonas viridis*, in reaction center solution and in reaction center crystals are reported. In solution and in the crystal a sign reversal of the F-ODMR signals is observed by variation of the wavelength of detection. The signs of the signals are interpreted with the Vredenberg-Duysens relation.

1. Introduction

The triplet state of photosynthetic reaction centers has been used in the past with noticeable success as an internal probe for the geometric and electronic structure of the complexes. Valuable information about binding conditions between the chlorophylls themselves and the protein has been obtained and the energy transport between antenna and reaction centers (RC) was investigated [1–8].

It is in these RCs, where after light excitation or the arrival of an antenna exciton, the photosynthetic charge separation is induced. This electron transfer can be blocked at the quinone by chemical reduction. The result is an effective population of the above-mentioned triplet state *via* the radical-pair-mechanism [9–10].

Optically detected magnetic resonance (ODMR) in zero field combines high sensitivity with high spectral resolution. Because Bacteriochlorophyll shows no phosphorescence, ODMR must be performed on either fluorescence (F-ODMR) or absorption (A-ODMR). In the last years a lot of ODMR-experiments were reported on RC-preparations and whole cells of various photosynthetic bacteria [11–14]. By site-selection techniques *e.g.* EEDOR it was possible to detect more than one triplet state in the same photosynthetic units [15–17].

The differences in the signs of the signals, detected on the fluorescence of the antenna pigments and on the fluorescence or absorption of the RC-P_{865} respectively were explained by energy transfer between antenna and RC, the Vredenberg-Duysens (VD)-relation [19, 14, 15, 18]. In the case of *Rhodopseudomonas viridis*, where the antenna is about 270 cm^{-1} lower in energy than the RC, contradictory results were published concerning the sign reversal [20, 21].

In this contribution we report on F-ODMR experiments, detecting the ODMR-transitions at different wavelengths on the fluorescence of whole cells, RCs in solution and RC-crystals, which prove, that the VD-relation holds in the case of *Rhodopseudomonas viridis*.

2. Materials and Methods

In our experiments we used a *reaction center preparation* from *Rhodopseudomonas viridis*, which was a kind gift of Dr. R. J. Cogdell. The RCs were prepared according to [22]. The preparation was reduced with 50 mM ascorbate at pH 8 under nitrogen atmosphere. The samples were diluted with Tris pH 8 and 60% glycerol to form a clear glass at low temperatures. The *RC-crystals* were a kind gift from Dr. J. Norris. They were stored under liquid nitrogen until usage. Their reduction was performed by ascorbate which was incorporated in the sucrose solution in which the crystals were stored [23]. Reduction of *whole cells* was performed with 5 mM *o*-phenanthroline to block the electron transfer and with 100 mM ascorbate to reduce the primary donor after light excited electron transfer [24]. The *optical density* of the solute samples was about 1 at the long wavelength band (1 cm cuvette). Quartz tubes with an inner diameter of 2 mm were filled to about 5 mm.

Reprint requests to Prof. Dr. H. C. Wolf.

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All F-ODMR experiments were performed at a temperature of 1.2 K. The experimental set-up is described elsewhere [25]. Additionally, we used for the optical excitation at 607 nm a cw dye laser system (Spectra-Physics Mod. 164-07 and Mod. 375).

We want to emphasize that ODMR of these preparations could only be seen in a good signal to noise ratio by proper reduction. Dithionite, which is commonly used as reducing agent for bacteriochlorophyll *a* containing bacteria is not very good for *Rhodopseudomonas viridis*, because it not only reduces the primary acceptor quinone but as well the bacteriopheophytin of the electron transport chain. This causes a bleaching of the triplet states which one wants to observe [26].

3. Experimental Results

Fig. 1 shows the *fluorescence spectra* of reduced whole cells and reaction centers in solution as well as of crystallized RCs. Their emission maxima are located at 1063 nm, 1013 nm and 1033 nm, respectively. In preparations of RCs one finds besides the main emission band additionally a weaker band at shorter wavelengths which is located at 954 nm in isolated RCs and hardly remarkable at 940 nm in the RC-crystal.

F-ODMR experiments were performed, monitoring the main fluorescence band of all three samples at different wavelength regions as can be seen in Figs. 2–4.

The resonance frequencies of the D − E transition and the D + E transition in *reduced whole cells* are 354 MHz and 580 MHz respectively and are independent of the wavelength of detection (Fig. 2). The *zfs* parameters, calculated from the resonance frequencies are tabulated in Table I. The resonance transitions are inhomogeneously broadened to a FWHM of 12–14 MHz. Holes can be burned into these lines with a half width of about 5 MHz.

Following the F-ODMR signals of RC-preparations along the wavelength of detection from

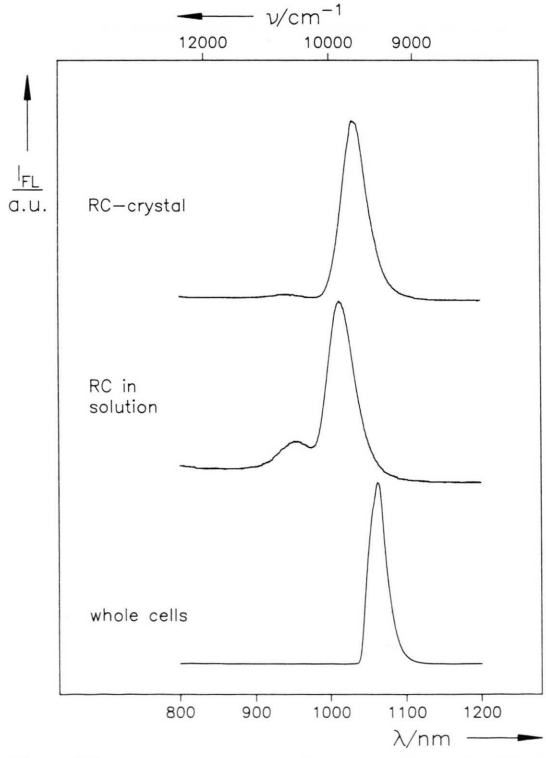

Fig. 1. Fluorescence spectra of whole cells and of RCs in solution and in crystal at $T = 1.2$ K. Excitation wavelength was 607 nm. The spectra are normalized to the same height of the main emission band in the near infrared.

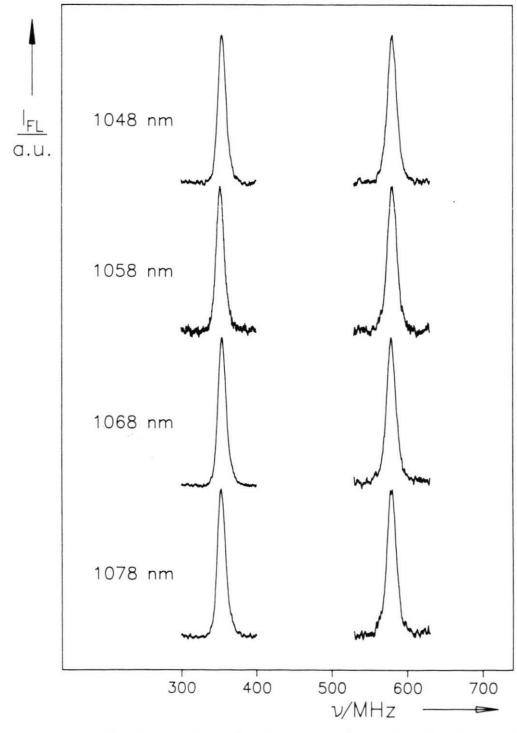

Fig. 2. F-ODMR signals from reduced whole cells as a function of the wavelength of detection which is written at the left of the spectra. $T = 1.2$ K. The spectra are normalized to the same heigth.

1052 nm to 972 nm, as shown in Fig. 3, one recognizes a sign reversal from positive to negative amplitude in both, the D + E and the D − E regions. At the long wavelength tail of the emission band, the resonance frequencies are located at 371 MHz and 618 MHz, at the short wavelength side at 366 MHz and 611 MHz. The correspomding D and E values are tabulated in Table I. Besides the somewhat higher D and E values, as compared with whole cells, we find a very large broadening of the D + E transition with a FWHM of 30 − 40 MHz. The half width of holes which can be burned in this signal is about 8 MHz.

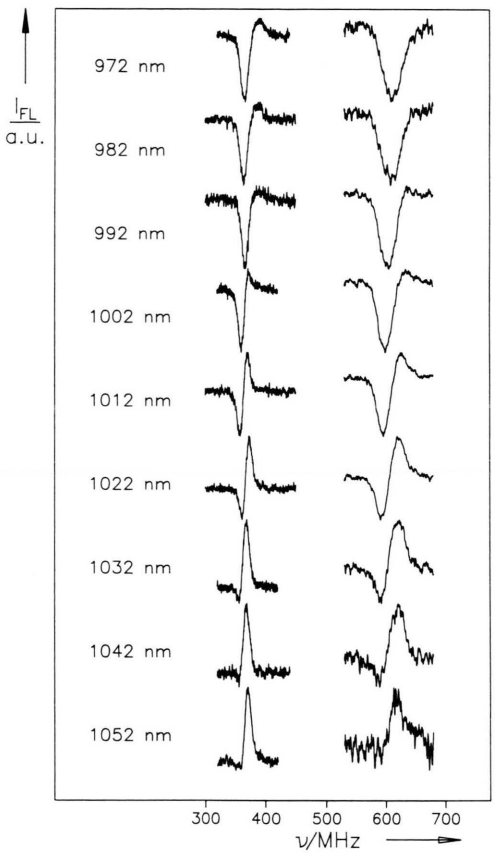

Fig. 3. F-ODMR signals of reaction centers in solution as a function of the wavelength of detection which is written on the left of the spectra. $T = 1.2$ K. The spectra are normalized to the same peak-peak distance.

Figure 4 represents ODMR signals of the *RC-crystal* at different wavelengths. The values for D − E and D + E are 360 MHz and 601 MHz, respectively. For the corresponding D and E values, see Table I.

The inhomogeneous linewidth of the two signals is between 12 and 16 MHz, which is in good agreement with the signals in whole cells. Interestingly, one can observe a sign reversal of the F-ODMR signals as well as in the solution by vary-

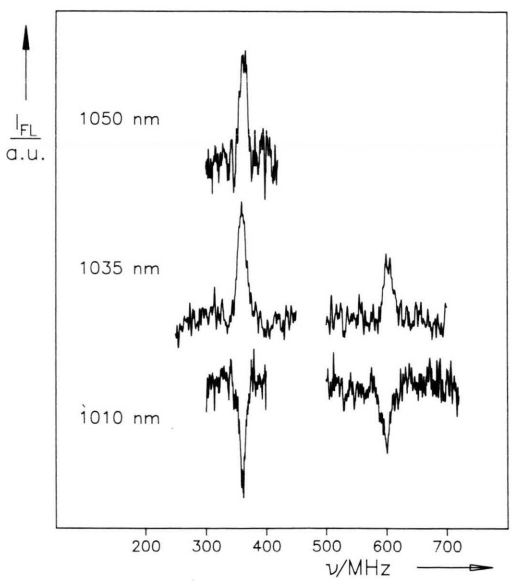

Fig. 4. F-ODMR signals of a RC-crystal as a function of the wavelength of detection. $T = 1.2$ K.

Table I. Comparison of the *zfs* parameters of the RC-triplet state, measured in whole cells, in RC solution and in RC-crystals with the values obtained by Hoff *et al.* in whole cells by F-ODMR and in RC solution by A-ODMR. [a] In Ref. [20] positive and negative signs are given.

	D	E	Inhom. FWHM	Hole FWHM	Sign	Ref.
whole cells	156.2	37.8			+	[20]
	155.8	37.7	14	5	+	this work
RC-solution	160.3	39.7			(−) [a]	[20]
at 980 nm	162.9	40.9	40	8	−	this work
at 1050 nm	164.9	41.2	30		+	this work
RC-crystal	160.3	40.2	16		+/−	this work

ing the wavelength of detection. In contrast to the case in solution, the positive and the negative signals show the same resonance frequencies. One should mention that the F-ODMR signals of the crystals are very weak. However, we experienced that the signals became better the longer we measured the crystal.

4. Discussion

Optical spectra

The emission band of reduced whole cells with its maximum at 1063 nm is due to the bulk antenna complexes of *Rhodopseudomonas viridis* as can be shown by fluorescence excitation spectra [27]. This is in good agreement with fluorescence data from Hoff [20] and Parson [28].

The emission maximum of the RC-P_{990} primary donor is invisible in whole cells because of its low fluorescence quantum efficiency [29] compared to the efficiency of antenna bacteriochlorophyll emission [30].

In RCs in solution we find the RC-P_{990} emission maximum at 1013 nm with a weaker band at 954 nm. It can be shown by excitation spectroscopy [27] that this weaker band is due to somehow denatured RCs. We find this band also very weakly in RC-crystal-preparations at 940 nm. The red shift of the P_{990} emission from 1013 to 1033 nm in RC-crystals as compared to solution it interpreted as due to reabsorption of the fluorescence. This seems reasonable because the optical density of the crystal is very high as compared to solution.

Zero field splitting

We interprete our results in accordance with the results obtained by Hoff *et al.* with F-ODMR and A-ODMR. The D and E values which were calculated from the measured resonance frequencies are compared with those observed previously by Hoff in whole cells with F-ODMR and in RC-preparations with A-ODMR [20]. There is very good agreement in the case of whole cells between our values and those of Hoff (see Table I). In RC-preparations we find two triplet states with somewhat higher D and E values. By A-ODMR Hoff found a similar rise in D and E. This may be due to the isolation procedure as Hoff pointed out [20]. The triplet state of the primary donor dimer is very sensitive to conformational changes that may be induced by the removal of the antenna complexes from the RCs. The discrepancy between our D and E values and those found by Hoff with A-ODMR can be explained by the fact that with F-ODMR we see another distribution of sites than with A-ODMR, *i.e.* those sites with maximal fluorescence quantum yield [18]. In RCs in solution we find a higher variability of different sites as can be seen by the larger line broadening of the resonance transitions with respect to whole cells.

In the RC-crystal we find nearly the identical D and E values as found by Hoff in solution with A-ODMR. The inhomogeneous linewidth is about the same as in whole cells. This reflects the regular composition of the crystal with only a small variety of sites.

Energy transfer

According to the VD-relation, describing the singlet energy transfer between antenna and RC [19], a bleaching of the RC singlet ground state absorbance causes an increase in fluorescence of the antenna complexes, provided that energy transfer from antenna to RC is possible. In the case of bacteriochlorophyll *a* containing bacteria *e.g.* *Rhodopseudomonas sphaeroides*, the VD-relation was applied to explain the sign reversal of the ODMR signals of P_{865}, detected *via* the fluorescence of the antenna in whole cells with respect to direct detection of the P_{865} emission in RC-preparations [14, 15].

In our present case, we have to pay attention to the fact that in *Rhodopseudomonas viridis* the antenna fluorescence is located at longer wavelengths than the RC-emission. The energy difference between P_{990} absorption and the long wavelength band of the antenna is about 270 cm^{-1} [31]. This energy barrier can easily be overcome at room temperature with thermal activation of the antenna excitons. At 1.2 K one should not observe such an energy transfer any more and the VD-relation should not be applicable.

When we compare the F-ODMR signals of *whole cells*, measured *via* the antenna emission (Fig. 2) with the F-ODMR signals of *RC-preparations* (Fig. 3) we find clearly a sign reversal. Where the sign in whole cells is positive *i.e.* an increase in fluorescence, the sign in the RC-preparation is negative, provided we observe at the short wave-

length side of the RC-emission. If we observe at the long wavelength tail we find a positive signal as in whole cells with slightly different *zfs* values. A similar *detection wavelength dependent* sign reversal of F-ODMR signals was reported in *Rhodopseudomonas sphaeroides* [15, 18] and explained with the assumption that in all RC-preparations there is a small amount of antenna bacteriochlorophyll (less than 1%) which contributes considerably to the whole emission because of the higher fluorescence quantum yield. The magnetic resonance of the RCs is now observed directly on the P_{890} emission or *via* the contaminating antenna pigments which causes a sign reversal according to the VD-relation.

The same arguments fit very well to the case of RC-preparations of *Rhodopseudomonas viridis*, if we take into account that the antenna emission is lower in energy. So we can ascribe the short wavelength part of the whole RC emission to fluorescence from P_{990} and the long wavelength part to fluorescence from contaminating antenna pigments which are energetically coupled to RCs.

This demonstrates that the signs of the ODMR signals in *Rhodopseudomonas viridis* can be explained by the VD-relation and the assumption, that we have in the RC-preparation contaminating antenna complexes, which are energetically coupled to RCs. In the past, there was some confusion concerning the sign of the absorbance detected ODMR signals [20, 21]. From our point of view, we would expect a negative A-ODMR signal *i.e.* a decrease in absorbance when measuring RC-preparation, as was reported in [21]. This would be consistent with our F-ODMR results and the comparable case of bacteria which contain bacteriochlorophyll *a*.

As a consequence, even at low temperatures energy transfer between antenna and RCs should be effective. This unusual uphill energy transfer is in our opinion explained by the very broad homogeneous linewidth of the singlet energy levels of about $200\,cm^{-1}$, according to FMDR data on *Rhodopseudomonas sphaeroides* [15]. This broad linewidth gives a small overlap between the antenna emission band and the RC absorption band.

Turning our attention now to the F-ODMR data of the *RC-crystal*, we find a similar sign reversal by variation of the detection wavelength. This seems strange, because one would not expect contaminating antenna in a *RC-crystal*. If we drop the assumption with those contaminants, we have to state nevertheless that we observe an inhomogeneous emission. That means, we have to attach the negative signal to another emitter with an existing energetic coupling to the pigment that contains the observed triplet state. On the other hand, it must be mentioned that the observed crystal fluorescence is only surface fluorescence. We cannot exclude that the surface of the crystal being in contact with the solvent is different from the bulk.

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