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LOW TEMPERATURE EXCITATION AND EMISSION SPECTROSCOPY OF THE PHOTOSYNTHETIC BACTERIA *RHODOPSEUDOMONAS SPHAEROIDES* 'WILD-TYPE' STRAIN ATCC 17023

G.H. KAISER, J. BECK, J.U. VON SCHÜTZ and H.C. WOLF

Physikalisches Institut, Teil 3, der Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80 (F.R.G.)

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Summary

Prompt and delayed emission of *Rhodopseudomonas sphaeroides* 'wild-type' strain ATCC 17023 was measured in the spectral range 550—950 nm at 1.7 K.

The broad emission spectra could be resolved into separate bands by excitation spectroscopy. Through a comparison of the separated excitation and emission spectra of the present study with absorption and fluorescence spectra of photosynthetic pigments given in the literature, the emitting species could be identified. Beside the well-known pigments bacteriochlorophyll (BChl), bacteriopheophytin (BPh) and carotenoids, additional pigments could be detected. In the visible and near-infrared range, the fluorescence of pigments could be observed which were produced by the biosynthesis of BChl in the cells. Further, fluorescence bands at 753 and 813 nm are interpreted as originating from BPh (*F*-753) and BChl (*F*-813).

Delayed emission signals between 700 and 800 nm were attributed to a metalloporphyrin phosphorescence. Surprisingly, the excitation spectra of the delayed BChl emissions measured at 872 and 912 nm showed the same excitation spectra as the short-wavelength emission, typical of metalloporphyrins. This is a strong indication of an energy transfer between metalloporphyrins and BChl.

Introduction

Photosynthetic bacteria exhibit weak prompt and delayed emissions in the visible [1,2] and the near infrared [3—6] regions.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

The infrared fluorescence of BChl *a*-containing purple bacteria is emitted almost exclusively by the antenna BChl which absorb at the longest wavelengths [7]. In *Rhodopseudomonas sphaeroides*, three different BChl components are detectable in the antenna systems. They are denoted by *B*-800, *B*-850 and *B*-870 using their absorption band positions in the red [8]. At room temperature, the excitation energy is transferred effectively from BChl-antenna complexes absorbing at short wavelengths to BChl-antenna complexes absorbing at long wavelengths [7]. Therefore, the fluorescence originates mainly from the BChl complex *B*-870 with a broad maximum at 895 nm [3]. Shoulders on the short-wavelength side of this fluorescence maximum indicate weak emissions from *B*-800 and *B*-850 [3,8].

Cooling down to 77 K results in a narrowing and a red shift of the infrared bands of the BChl-antenna complexes [3]. Due to the fact that in *Rps. sphaeroides B*-870 is more strongly red shifted than *B*-850, a second fluorescence band at about 878 nm was expected but has not been resolved thus far [3,4]. The delayed emission of photosynthetic bacteria coincides at least in the infrared region with the bands of the prompt fluorescence [5,6]. It is generally assumed that this delayed fluorescence is caused by a reversal of photochemistry which results in a reexcitation of the antenna pigments [7]. A delayed emission (ms time range) of *Rps. sphaeroides* in the visible region was associated following Arata et al. [6] with Mg-protoporphyrin IX, a compound produced by BChl biosynthesis.

The aims of this work were the investigation of the prompt and delayed emission of *Rps. sphaeroides* in the visible and infrared spectral range, the separation of the superimposed bands and the identification of additional fluorescent pigments besides the known emissive compounds. To achieve a better separation of the emissive bands, all experiments were carried out at low temperatures (1.7 K) at which generally the linewidth of electronic transitions is lowered. In addition, excitation spectroscopy was applied in order to attribute the detected emission bands to the emitting molecules or complexes.

Materials and Methods

Growth of bacteria. Rps. sphaeroides 'wild-type' strain ATCC 17023 was cultured anaerobically under incandescent light at 30°C for 3—5 days in a medium described by Cohen-Bazire et al. [9]. 1 g casein hydrolysate per l was added to the medium. The bacterial cells were cultivated in closed 0.5-l or 1-l glass bottles under gentle stirring with magnetic bars. Freshly inoculated cultures were incubated for 1 day at 30°C in the dark.

Sample preparation. The cells were centrifuged and washed three times with phosphate buffer (pH 7) under a nitrogen atmosphere. The phosphate buffer contained 24 mM KH_2PO_4 and 34 mM K_2HPO_4 and was kept anaerobic by bubbling with nitrogen gas. An addition of an enzymatic system of 70 mM glucose and 300 mg/l glucose-oxidase was used to trap oxygen [2]. The absorbance of the sample was adjusted to 1 at 590 nm (1-cm cuvette) by dilution with a mixture of glycerol and phosphate buffer (1 : 1, v/v). This suspension was placed into a thin-walled tube of suprasil (Schott, inner diameter 2.2 mm) and quickly cooled to 77 K in the cryostat.

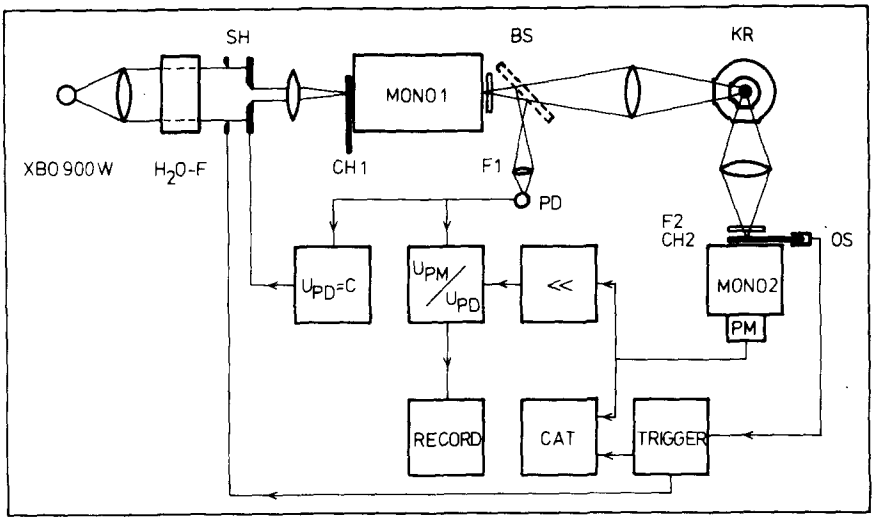


Fig. 1. Experimental arrangement for the measurements of the prompt fluorescence, the delayed emission and of the excitation spectra. XBO 900W, xenon high pressure lamp XBO 900W; H_2O-F, water-filter, 10 cm; SH, shutter; MONO1, double monochromator; BS, beam splitter; KR, He-cryostat; CH1/CH2, chopper 1/chopper 2; F1/F2, filter 1/filter 2; PD, photodiode; OS, optical switch; MONO2, monochromator; PM, photomultiplier; U_{PM}, photomultiplier current; U_{PD}, photodiode current; U_{PM}/U_{PD}, divider; U_{PD} = C, comparator; Record, *X-t*-recorder; CAT, computer of average transients.

Experimental methods. Prompt and delayed emission of *Rps. sphaeroides* was measured at 1.7 K using a spectrofluorimeter (Fig. 1). The excitation light source was a xenon high-pressure lamp XBO 900 W (Osram).

For measuring the fluorescence, the excitation light was passed through a water-filter, a double monochromator (Spex Mod. 1672 Doublemate) and appropriate cut-off filters (F1) before being focused by quartz lenses onto the sample in the variable-temperature He-cryostat. The fluorescence of the sample was monitored using suitable filters (F2) and a 25-cm monochromator (Jarrell Ash). The detector for the spectral range between 500 and 850 nm was a cooled EMI 9658 photomultiplier with modified S-20 cathode. Above 850 nm, a nitrogen-cooled EMI 9684 photomultiplier with a S-1 cathode was used. The photomultiplier current was amplified with a picoammeter (Keithley 414A). The amplified signal was recorded on a *X-t* recorder (HP Model Moseley 7100 BM).

The separation of delayed and prompt emission was achieved by two crossed choppers (CH1, CH2) [10]. The time resolution (dead-time between on and off) was 0.3 ms. Decay kinetics of the delayed emission were analysed by a computer of average transients (HP-CAT 5480A) to increase the signal-to-noise ratio. For this purpose a shutter (Compur electronic 5FS) as well as the trigger of the computer of average transients were synchronised to the choppers.

The excitation spectra in the region between 300 and 950 nm were measured at constant excitation light intensity. For this purpose, part of the excitation light was diverted with a beam splitter placed behind the double monochromator and focused onto a photodiode (UDT, Pin 10, ultraviolet-enhanced). The

current of the photodiode was amplified in a comparator which generated a regulation signal for a shutter placed in the excitation path. With this arrangement, the excitation intensity could be kept constant, sweeping in the whole spectral range although the intensity of the lamp varies drastically. An additional refinement of the regulation was achieved by the division of the photomultiplier current by the signal of the photodiode. These normalized fluorescence signals were plotted with a *X-t* recorder. The spectral sensitivity of the optical detection was determined using the standard spectrum of a tungsten lamp. The emission spectra given in Fig. 2 are corrected with the calibration factors. On the other hand, all excitation spectra are corrected automatically as explained above.

Distortion of the fluorescence emission by reabsorption effects. This was tested via the ratio of the intensities of the infrared bands of the BChl fluorescence (*F*-876/*F*-916). These wavelengths were chosen because of the strongly different absorption coefficients ($A_{876}/A_{916} \approx 6$). In samples with low cell concentrations (absorbance at 590 nm lower than 1 in a 1-cm cuvette), this ratio is no longer concentration-dependent and the spectra are no longer distorted by reabsorption effects. This is not surprising if we take into account that our effective sample thickness is less than 1 mm due to the geometry used in the experiment (sectorial excitation).

Results and Discussion

Fig. 2 shows the prompt fluorescence of *Rps. sphaeroides* at 1.7 K in the spectral range between 550 and 950 nm using two different excitation wavelengths (376 nm, dotted line and 428 nm, continuous line; both curves are normalized to equal height at 916 nm). Fluorescence peaks are detectable at 916, 876, 813, 753, 666, 625 and 597 nm. The dependence of the relative intensities of the bands on excitation wavelength is strongest in the spectral

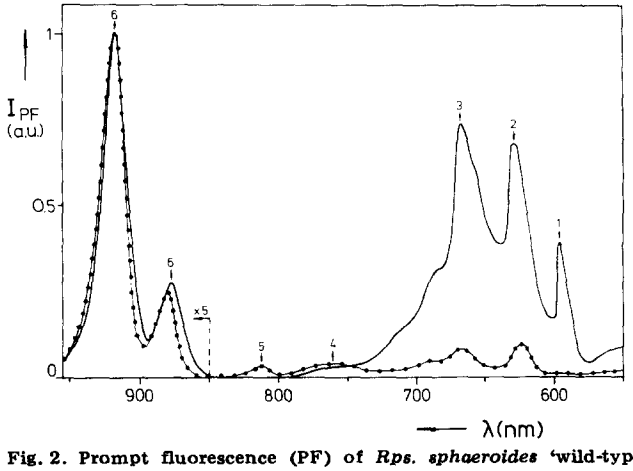


Fig. 2. Prompt fluorescence (PF) of *Rps. sphaeroides* 'wild-type' at different excitation wavelengths (376 nm, dotted line; 428 nm, continuous line). Both curves are normalized to equal height at 916 nm. Above 850 nm, the spectra must be multiplied by a factor of 5. Excitation, bandpass 5 nm; monitoring monochromator, bandpass 1.5 nm; filters F2, above 700 nm; 2 × KV550 (Schott), below 700 nm: 2 × KV450 (Schott). a.u., arbitrary units.

region between 580 and 820 nm, whereas the ratio of the fluorescence bands *F*-916/*F*-876 is nearly independent of excitation wavelength.

The strong dependence of the monitored emission on excitation wavelength was utilized to identify the origins of the fluorescence bands. In the following, beginning with the short wavelength bands, the excitation spectra of the emission bands are described. It is assumed that fluorescence bands with similar excitation spectra are emitted by the same pigment.

In Fig. 3, the excitation spectrum of the fluorescence band *F*-597 (dotted line) is shown. Two bands are observed: an intensive Soret band at 428 nm and a band at 560 nm which is about a factor of 10 weaker. Monitoring optically at 656 nm, an excitation spectrum of similar structure could be measured (Fig. 3, continuous line). Besides, a somehow broader Soret band at 426 nm and further, weak bands at 507, 540, 561, 599, 606 and 627 nm were detected. The bands at 507, 540, 606 and 627 nm are due to additional pigments emitting at 656 nm (*F*-666, see below).

From the agreement of the Soret band at 426 nm and the band at 560 nm, the following explanation can be given: the pigment which is fluorescing at 597 nm has an additional but weaker fluorescence maximum at 656 nm. The excitation spectrum which can be correlated to this pigment shows an intense Soret band at 428 nm and two weaker bands at 560 and 599 nm.

Silberstein et al. [1] reported a similar fluorescence of *Rhodospirillium rubrum* at room temperature. They observed a fluorescence maximum at 596 nm and an additional band at 654 nm. The corresponding excitation spectrum has a strong Soret band at 422 nm and additional bands at 470, 554 and 592 nm.

Arata et al. [2] observed, at room temperature, a delayed emission of *Rhs. rubrum* and *Rps. sphaeroides* in the time scale of ms with similar spectral properties. They associated this delayed light with Mg-protoporphyrin IX or a similar compound. Mg-protoporphyrin IX is known to be a precursor in the

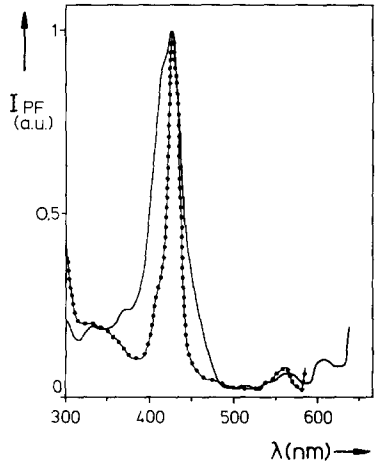


Fig. 3. Excitation spectra of *F*-597 (dotted line) and *F*-656 (continuous line). Excitation, bandpass 5 nm; monitoring monochromator, bandpass 1.5 nm; filters F2, 2 × RG590 (Schott), *F*-597; 2 × RG645 (Schott), *F*-656. PF, prompt fluorescence; a.u., arbitrary units.

TABLE I

FLUORESCENCE PROPERTIES OF Mg-PROTOPORPHYRIN IX AND OF Mg-PROTOPORPHYRIN IX MONOMETHYL ESTER COMPARED WITH THE FLUORESCENCE PROPERTIES OF *F*-597

Compound	Emission bands	Excitation bands	Ref.
Mg-protoporphyrin IX	*DE*-600, *DE*-650	410, 550, 590	[2]
Mg-protoporphyrin IX monomethyl ester	*F*-600, *F*-658	424, 556, 594	[12]
	F-597, *F*-656	428, 560, 599	This work

biosynthesis of BChl [11]. Rebeiz et al. [12] reported the synthesis and accumulation of metalloporphyrins in etiolated cucumber cotyledons during greening. One of the metalloporphyrins identified by them is Mg-protoporphyrin IX monomethyl ester. Mg-protoporphyrin IX monomethyl ester-enriched cotyledons have fluorescence maxima at 600 and 658 nm. The excitation spectra of these emissions show Soret maxima at 424 nm and α- and β-excitation bands at 594 and 556 nm [12].

In Table I, the reported spectroscopic data of several authors are summarized. The significant agreement of these data leads to the conclusion that this pigment observed at low temperatures in fluorescence is Mg-protoporphyrin IX or Mg-protoporphyrin IX mono(methyl) ester which is identical in the spectroscopic data [13].

Fig. 4 shows the excitation spectrum of the fluorescence band *F*-626 (dotted line) measured at 1.7 K. Worth mentioning is the occurrence of two Soret bands at 409 and 449 nm. Weak bands which are at least one order of magnitude smaller are also detectable at 506, 540 and 573 nm.

An excitation spectrum of quite similar structure can be observed at 688 nm (Fig. 4, continuous line). The Soret bands coincide with those of *F*-626 as well as the weak bands at 508, 542, 568 and 574 nm. Additional bands at 514, 621

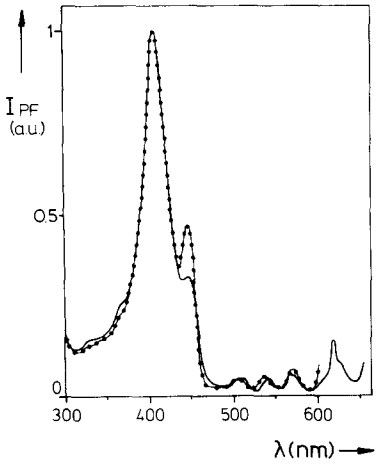


Fig. 4. Excitation spectra of *F*-626 (dotted line) and *F*-688 (continuous line). Excitation, bandpass 5 nm (*F*-626); 2 nm (*F*-688); monitoring monochromator, bandpass 3 nm (*F*-626), 1.5 nm (*F*-688); Filters F2, 2 × RG610 (Schott), *F*-626; 2 × RG665 (Schott), *F*-688.

TABLE II

ABSORPTION BANDS OF PROTOPORPHYRIN IX-DIMETHYL ESTER AND OF Mg-2,4-DIVINYL-PHAEOPORPHYRIN a_5 MONOMETHYL ESTER COMPARED WITH THE EXCITATION BANDS OF *F*-626

Compound	Absorption bands	Ref.
Protoporphyrin IX dimethyl ester	408, 505, 540.5, 578, 633	14, 15
Mg-2,4-divinylphaeoporphyrin a_5 monomethyl ester	438, 573, 624	16
	409, 449, 506, 540, 573, 621 (excitation bands)	This work

and 629 nm are monitored. The excellent conformity of the excitation spectra, measured at an observation wavelength of 626 and 688 nm allows the statement that both emissions originate from the same pigment *F*-626 with excitation bands at 409, 449, 506, 540, 573 and 621 nm. This excitation spectrum is also typical for metalloporphyrins (strong Soret band and weaker bands in the visible region). The presence of two Soret bands is unusual and points to the possibility that at 626 and 688 nm, the fluorescence of two different pigments is observed. This explanation is supported by the fact that at several samples, a splitting of the fluorescence band *F*-626 was detectable. Therefore, we attempt to explain the excitation spectra of *F*-626 with a superposition of the excitation spectra of two different pigments fluorescing at 626 and 688 nm.

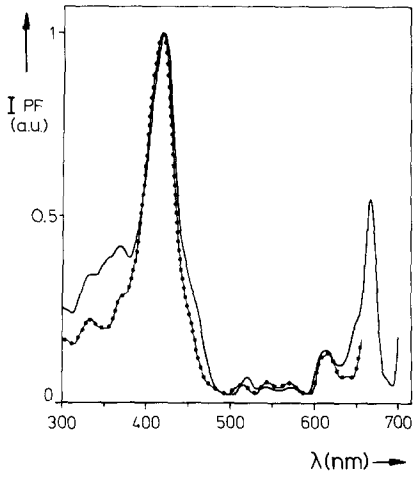
It is assumed that the detected fluorescence bands are produced by protoporphyrin IX and the protochlorophyll-like compound Mg-2,4-divinylphaeoporphyrin a_5 monomethylester. Both pigments are also BChl precursors. Table II shows the absorption bands of these compounds.

The wavelength of the mesoporphyrin fluorescence maximum (a protoporphyrin IX-like compound) was determined by Gurinovich et al. [17] to be at 617 nm. From the difference between both long-wavelength absorption bands of protoporphyrin IX, the wavelength of the long-wavelength fluorescence maximum can be estimated roughly to be about 680 nm.

According to the structural similarity between Mg-2,4-divinylphaeoporphyrin a_5 monomethyl ester and protochlorophyll, it can be assumed that the fluorescence of this pigment corresponds to the fluorescence of protochlorophyll which is known at room temperature to have its maximum between 626 and 631 nm (solvent-dependent) [17]. In addition, protochlorophyll shows a broad long-wavelength maximum at about 680 nm.

The agreement of the absorption and fluorescence properties of these compounds with the measured excitation and fluorescence spectra of *F*-626 and *F*-688 is relatively good. Therefore, we believe that the fluorescence bands *F*-626 and *F*-688 are produced by the fluorescence of the pigments protoporphyrin IX and Mg-2,4-divinylphaeoporphyrin a_5 monomethyl ester.

The observed excitation spectra of the fluorescence band *F*-666 at 1.7 K is plotted in Fig. 5 (dotted line). A strong Soret band at 417 nm and several weak bands at 510, 542, 566, 607, 614 and 637 nm are detected. An excitation spectra of similar excitation wavelength dependence can be observed at 720 nm also



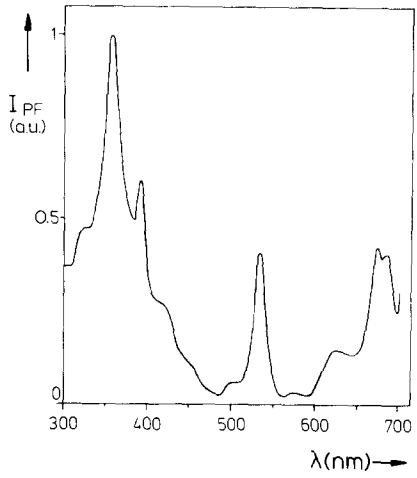


Fig. 5. Excitation spectra of *F*-666 (dotted line) and *F*-720 (continuous line). Excitation, bandpass 5 nm; monitoring monochromator, bandpass 3 nm; filters F2, 2 × RG665 (Schott), *F*-666; 2 × RG715 (Schott), *F*-720.

Fig. 6. Excitation spectra of *F*-753. Excitation, bandpass 5 nm; monitoring monochromator, bandpass 1.5 nm; filters F2, 2 × RG715 (Schott).

(Fig. 5, continuous line). In this case, an intense Soret band at 419 nm is detected and additional weak bands at 519, 537, 569, 611 and 650 nm, as well as a relatively strong band at 666 nm, are visible. We conclude from the conformity of the excitation spectra measured at 666 and 720 nm, that the pigment fluorescing at 666 nm has a corresponding band at 720 nm. The excitation bands of this pigment are localized at 417, 510, 542, 566, 610 and 666 nm. Lascelles [16] published absorption spectra of a pigment (*P*-662) isolated from cultures of mutant strains of *Rps. sphaeroides*. She identified this pigment, a BChl precursor, tentatively as 2-devinyl-2-hydroxyethyl chlorophyllide. The absorption bands of this pigment are situated at 418, 430, 624 and 662 nm. To our knowledge, fluorescence spectra of this compound are not known. Therefore, from the similarity of the excitation spectra detected at *F*666 and *F*720 with the absorption spectrum of *P*-662, we conclude that the pigment observed in fluorescence is 2-devinyl-2-hydroxyethyl chlorophyllide.

The excitation spectrum (Fig. 6) of the fluorescence peak *F*-753 exhibits two intense bands in the ultraviolet region at 360 nm and at 393 nm. In addition, a relatively strong band at 535 nm in the visible and weak bands at 573 and 624 nm are observable. In the near infrared, a split band at 673 nm and at 678 nm was detected. Except for small band shifts, a comparison of this excitation spectrum with an absorption spectrum of BPh *a* in a solvent gives good agreement. At room temperature, the fluorescence maximum of BPh *a* in ether was estimated from curves given by Goedheer [19] to lie at 762 nm. The good correspondence between these data and our observations allows us to attribute the detected fluorescence band at 753 nm to BPh.

A further comparison can be achieved via the absorption spectrum of reaction centers of *Rps. sphaeroides* R-26. This spectrum shows at room temper-

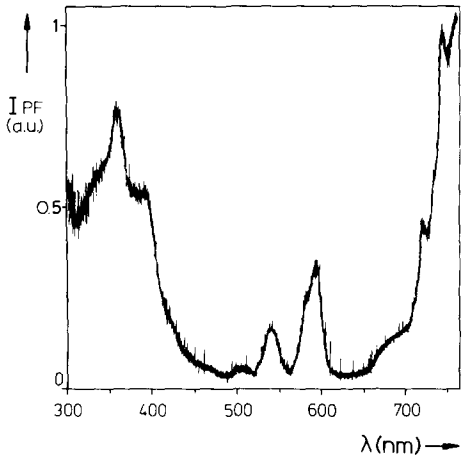


Fig. 7. Excitation spectra of *F*-813. Excitation, bandpass 5 nm; monitoring monochromator, bandpass 3 nm; filter F2, B-40-801 (Balzers).

ature two BPh absorption bands at 535 and 759 nm [20]. Cooling to 77 K splits the 535 nm absorption band of this protein-bound reaction center BPh into two bands of equal height with maxima at 530 and 542 nm [21,22].

The excitation peak of *F*-753 does not show a splitting at 535 nm. Therefore, we do not assume that the protein-bound reaction center BPh is the emitter of *F*-753. Possibly, the bacterial cells contain BPh as a contaminant, produced by denaturation of BChl.

In Fig. 7, the excitation spectrum of the fluorescence band *F*-813 at 1.7 K is plotted. It consists of two intense Soret bands at 360 and 394 nm, two relatively strong bands in the visible at 540 and 592 nm and two bands in the near infrared at 717 and 741 nm. This can be understood as a superposition of BPh and BChl excitation bands if we take into account the known absorption bands of BPh (363 nm, 533 nm) [23] and BChl-antenna complexes of *Rps. sphaeroides* (376 nm, 588 nm) [24] at room temperature.

A superposition of the long-wavelength shoulder of the BPh emission [19] and of the fluorescence of the *B*-800-antenna complexes which emit at 805 nm at room temperature [8], could be the source of the fluorescence band at 813 nm. This is, however, questionable, because in this case we would expect carotenoid bands (carotenoids are present in the antenna complex [24,25]) in the excitation spectrum which are not found.

The emission of *Rps. sphaeroides* possesses, at 1.7 K, two clearly separated maxima in the infrared at 876 nm and at 916 nm (Fig. 2). Within experimental error, the excitation spectra of these fluorescences are identical. For this, in Fig. 8 only the excitation spectrum of *F*-916 is shown. It is composed of an intense Soret band at 376 nm and additional bands in the visible and infrared (527, 544, 590, 770, 807, 860 and 896 nm). Between 440 and 500 nm and at 734 nm, weaker bands are observable. The excitation bands can be identified by comparison with absorption spectra of photosynthetic pigments.

At room temperature, Olson et al. [26] reported absorption bands of BChl complexes from *Rps. sphaeroides* at 376, 589, 800, 850 and 875 nm. Cooling

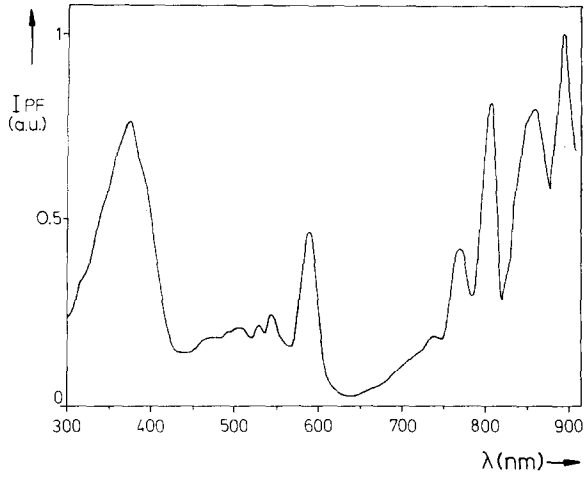


Fig. 8. Excitation spectra of *F*-916. Excitation, bandpass 5 nm; monitoring monochromator, bandpass 3 nm; filter F2, AL 909 (Schott).

to 77 K generally leads to a shift of the long-wavelength absorption bands to the red [3]. Therefore, it is assumed that the detected bands at 376, 590, 807, 860 and 896 nm are excitation bands of the BChl-antenna pigments.

Energy transfer plays an important role in the explanation of the excitation spectra of *F*-876 and *F*-916. Weak absorption peaks between 420 and 510 nm coincide with absorption bands of carotenoids which are present in BChl-antenna complexes [24] and from which it is known that the efficiency of the energy transfer is quite high (about 90% at room temperature [27]). Observed excitation bands at 527, 544 and 770 nm are in good agreement with absorption bands of protein-bound BPh [21]. For this an additional energy transfer between BPh and BChl-antenna complexes must be assumed.

However, BPh has a functional role only in the reaction centers of the bacterial photosystems. For that reason, the mechanism of such an energy transfer is not yet clear. A simple explanation is that also in the antenna systems protein-bound BPh can occur (as a contamination, perhaps by denaturation of BChl) and can transfer excitation energy to BChl.

Delayed emission of *Rps. sphaeroides* at low temperatures is about three to four orders of magnitude weaker than the prompt emission. The spectrum itself is similar to the spectrum of the fluorescence as far as just the spectral range between 800 and 950 nm is concerned. Maxima are observed at 810, 872 and 912 nm.

No similarity between prompt fluorescence and delayed emission is seen in the spectral range between 500 and 800 nm. The delayed emission in this range exhibits one intense band at 709 nm and weaker bands or shoulders at 623 and 756 nm.

Excitation spectra of the delayed emission are similar, independent of the wavelength monitored (709, 756 or 912 nm). An intense Soret band at 422 nm and weaker bands at 548 and 582 nm are detectable. No BChl-like excitation spectra could be measured as found monitoring the prompt fluorescence, but the observed excitation spectra are similar to the fluorescence excitation spec-

tra of simple metalloporphyrins (see Fig. 3). It is known that these compounds can show phosphorescence [17]. Because of the difference between prompt and delayed emission in the spectral range between 500 and 800 nm, we believe that in this range delayed emission consists of phosphorescence of a metalloporphyrin, probably a compound similar to Mg-protoporphyrin IX.

Although prompt fluorescence and delayed emission have similar spectral shapes in the spectral range between 800 and 950 nm, their excitation spectra are quite different. For this, we believe that excitation energy is transferred delayed onto the BChl-antenna complexes and these complexes then show a delayed fluorescence. We assume an energy transfer between the phosphorescent short-wavelength metalloporphyrins and the long-wavelength BChl-antenna complexes.

The decay kinetics of the delayed emission were not monoexponential. The half-times of the different bands of delayed emission varied between 40 (*DE*-709) and 8 ms (*DE*-912). Reduction of the sample with sodium dithionite enhanced the half-time of the short-wavelength emission (*DE*-709, 110 ms).

Concluding remarks

Fluorescence emissions of biological systems are generally difficult to interprete due to the large number of molecules emitting and the energy transfer processes between them. The complex emission spectra of *Rps. sphaeroides* is a superposition from all fluorescent pigments in the cells. A further selection of individual emission bands of different molecules or complexes was attempted using excitation spectroscopy. It could be shown by this method that the fluorescence between 590 and 950 nm originates from the following pigments, as indicated in Fig. 2. (1) Mg-protoporphyrin IX (monomethyl ester); (2) protoporphyrin IX, Mg-2,4-divinylphaeoporphyrin a_5 monomethyl ester; (3) 2-devinyl-2-hydroxyethyl chlorophyllide; (4) bacteriopheophytin; (5) bacteriopheophytin, bacteriochlorophyll and (6) bacteriochlorophyll-antenna complexes.

Surprisingly, the separation by excitation spectroscopy was so definite and unequivocal that many components of the BChl biosynthesis pathway could be identified without a special chemical preparation.

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