Biochimica et Biophysica Acta, 681 (1982) 359–364
Elsevier Biomedical Press

BBA 41143

PROMPT AND DELAYED FLUORESCENCE IN PIGMENT-PROTEIN COMPLEXES OF A GREEN PHOTOSYNTHETIC BACTERIUM

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(Received February 22nd, 1982)

Key words: Fluorescence; Excitation spectrum; Magnetic field; Bacterial photosynthesis; Pigment-protein complex; (Prosthecochloris aestuarii)

Excitation spectra were measured at 4 K of bacteriochlorophyll *a* fluorescence in reaction center containing pigment-protein complexes obtained from the green photosynthetic bacterium *Prosthecochloris aestuarii*. Excitation spectra for the longest-wave emission (838 nm) showed bands of bacteriochlorophyll *a*, carotenoid, and of a pigment with absorption bands at 670, 438 and possibly near 420 nm, which is probably identical to an unidentified porphyrin described in the preceding paper (Swarthoff, T., Kramer, H.J.M. and Amesz, J. (1982) Biochim. Biophys. Acta 681, 354–358). At room temperature the longest-wave emission is stimulated by a magnetic field, which indicates that at least part of the emission is delayed fluorescence brought about by a reversal of the primary charge separation. Below about 150 K no stimulation was observed. The excitation spectra for short-wave emission (828 nm) were very similar to the absorption spectrum of the isolated antenna bacteriochlorophyll *a*-protein complex, and showed bands of bacteriochlorophyll *a* only. This indicates that two forms of the antenna protein exist that are spectroscopically similar: a soluble form that is released by treatment with guanidine hydrochloride and a bound form that remains attached to the reaction center complex. The bands of the antenna complexes were weak in the excitation spectra of the 838 nm fluorescence, which indicates that the efficiency of energy transfer to the reaction center complex is low.

Introduction

In previous publications we have reported on the photochemical [1–4] and structural [5,6] properties of reaction center containing pigment-protein complexes derived from the green photosynthetic bacterium *Prosthecochloris aestuarii*. Data on the pigment composition of these complexes are given in an accompanying paper [7]. The fluorescence emission spectra of BChl *a* in the complexes were found to consist of two major emission bands, near 815–817 and 835–837 nm [6,8], whereas intact cells of green bacteria show only a BChl *a* band near 815 nm [9,10]. The longer-wave emission appears to be due to BChl *a* associated with the raction center [6]. Part of the emission at 815 nm is probably due to the water-soluble light-harvesting BChl *a* protein [11], since the emission spectrum of the isolated complex shows a major band at 816 nm [6,10,12]. Upon cooling, the band at 816 nm was replaced by one at 828 nm in all preparations tested [6]. The same is true for intact cells [6,13]. The relative heights of the bands in the emission spectra of the pigment-protein complexes were found to vary with the wavelength of excitation. Upon excitation at 670 nm, the most prominent band was at 836–838 nm, whereas excitation

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P-840, primary electron donor bacteriochlorophyll.

at 603 nm favored the emissions at 817 and 828 nm [6,8].

In order to obtain more information about the structural features underlying this phenomenon and about the pathways of energy transfer between the various pigments, we have measured action spectra of fluorescence and of the photochemical charge separation in various types of preparations from *P. aestuarii*, the results of which are reported in this paper. Experiments are also presented concerning the nature of the long-wave emission band.

Material and Methods

The bacteria were grown, and the pigment-protein complexes were prepared as indicated in the preceding paper [7]. The preparations were suspended in 10 mM phosphate and 10 mM ascorbate, pH 7.4, containing 40% sucrose. For measurements at low temperature, glycerol (50%, v/v) was added to prevent crystallization.

The apparatus and methods used to measure absorption and fluorescence emission and excitation spectra are described in Refs. 14 and 15. For measuring fluorescence spectra the analyzing monochromator was set at a half-bandwidth of 1.6 nm, while for the excitation spectra both the excitation and the analyzing monochromator were set at 3.2 nm. The spectra were scanned and plotted at 1 nm intervals. The excitation monochromator was equipped with Balzers Calflex B1/K1 and Schott UG 10 (4 mm) filters for the visible region of the spectrum and with Schott RG 665 (3 mm) and BG 26 (3 mm) filters for the infrared. The fluorescence spectra were corrected for the sensitivity of the photomultiplier and the transmission of the analyzing monochromator [14]. The excitation spectra were plotted as fluorescence intensity per incident quantum.

In order to avoid self-absorption of fluorescence, the fluorescence excitation and emission spectra were measured with dilute samples ($A \simeq 0.08$ at the main near-infrared maximum). Absorption changes due to photooxidation of P-840 were measured at 830 nm in a dual-beam spectrophotometer.

The magnetic field-induced emission was measured in a specially developed spectrophotometer, by analysis of the effect of a sinusoidally varying magnetic field (frequency 50 Hz) on the yield of fluorescence [16]. Changes in sensitivity of the photomultiplier (Dumont KM 2290, S1-type) due to the magnetic field were less than $2 \cdot 10^{-5}$. The emission was filtered through appropriate interference filters (bandwidth 15 nm) and a Schott RG 715 (3 mm) filter. The excitation light passed a Balzers Calflex-C and a Schott AL 606 or 679 nm interference filter; the intensities were 8 and 12 mW/cm^2, respectively.

Results and Interpretations

Low-temperaure excitation spectra

An emission spectrum of the pigment-protein complex, which contains about 75 BChl *a* molecules per reaction center [1], is shown in Fig. 1. The two near-infrared emission bands at 828 and 838 nm are due to BChl *a* [6]; the band at 674 nm may be related to the emission band at 674 nm earlier observed at room temperature [8]; its relative intensity varied for different preparations. The BChl *a* emission band at 815 nm that is observed in the room-temperature spectrum [8] disappears upon cooling below 40 K [6].

Fig. 2 shows fluorescence excitation spectra of the pigment-protein complex, measured at 4 K, for fluorescence emitted at 838 and 828 nm, respectively. The absorption spectrum (1 − T) is also

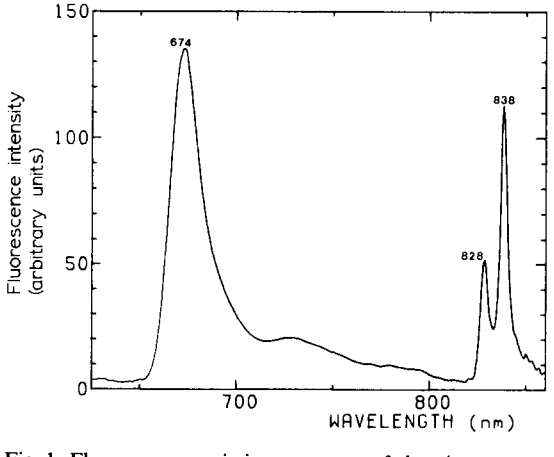

Fig. 1. Fluorescence emission spectrum of the pigment-protein complex at 4 K. Wavelength of excitation: 435 nm.

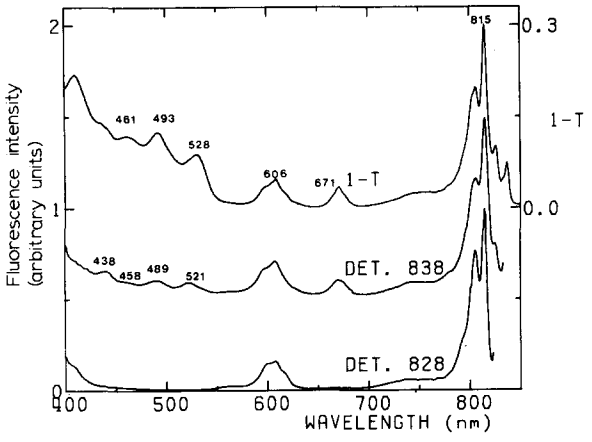

Fig. 2. Absorption spectrum (1 − transmittance, top) and excitation spectra for fluorescence emitted at 838 and 828 nm of the pigment-protein complex at 4 K. The spectra are displaced vertically in order to enhance clarity; the ordinate scale for the absorption spectrum is given at the right-hand side. The left-hand scale refers to the excitation spectra; the units are arbitrary and the spectra are normalized at their maxima. The excitation spectrum for the 838 nm fluorescence was shifted by 0.5 units. DET., detection wavelength.

shown. The two excitation spectra are clearly different. The first spectrum shows bands at 671 nm and in the carotenoid region, which are completely lacking in the second one. Moreover, near 600 and between 770 and 830 nm the spectra show different contributions by the Q_x and Q_y bands of BChl *a* that make up the absorption spectrum in these regions. The excitation spectrum of the fluorescence at 674 nm showed bands at 416 and 436 nm and weaker ones at 515 and 550 nm (Fig. 3). The

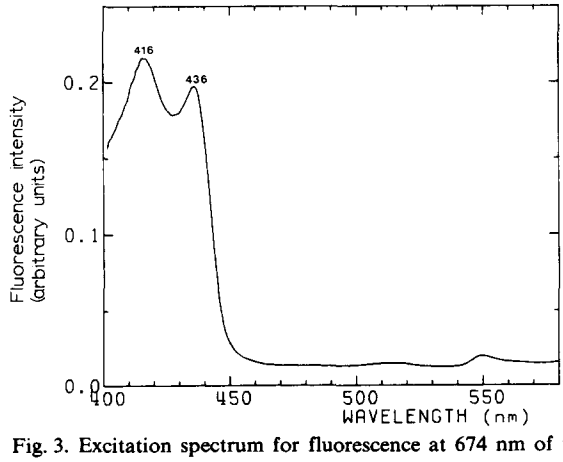

Fig. 3. Excitation spectrum for fluorescence at 674 nm of the pigment- protein complex at 4 K.

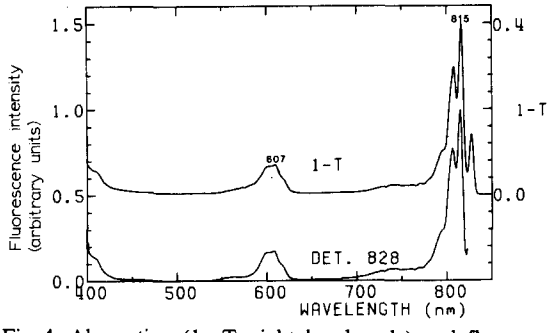

Fig. 4. Absorption (1 − T, right hand scale) and fluorescence excitation spectrum of the soluble antenna BChl *a* protein at 4 K. Fluorescence was detected at 828 nm.

relative height of these bands varied for different preparations; sometimes the band at 436 nm was weaker relative to the others, and an additional weak band at 480 nm could be observed in the action spectrum.

Absorption and excitation spectra of the water-soluble light-harvesting BChl *a* protein are shown in Fig. 4. The shape of the excitation spectrum is almost indistinguishable from that of the 828 emission in the pigment-protein complex. On the other hand, comparison of Figs. 2 and 4 shows that the characteristic bands of the light-harvesting complex are quite weak in the excitation spectra for the 838 nm emission, as can be most clearly seen at 620, 805 and 823 nm. Since the long-wave band (838 nm) appears to be emitted from the vicinity of the reaction center, as will be discussed below, this indicates that the efficiency of energy

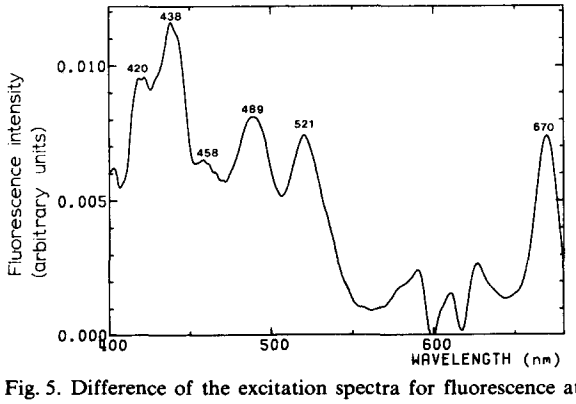

Fig. 5. Difference of the excitation spectra for fluorescence at 838 and 828 nm of the pigment-protein complex. The spectra were normalized at 599 nm before subtraction.

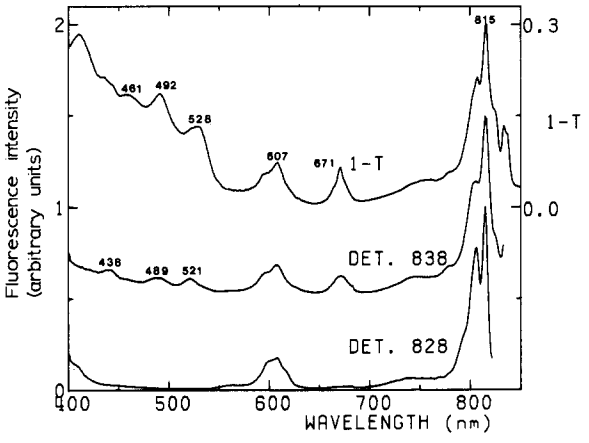

Fig. 6. Absorption (1−T) and fluorescence excitation spectra of the reaction center pigment-protein complex at 4 K, plotted as for Fig. 1.

transfer from the light-harvesting BChl *a* complex to the reaction center complex is low (less than 30%). The fluorescence yield at 828 nm per absorbed quantum at 606 nm was only about 70% higher for the BChl *a* protein than for the pigment-protein complex, which also suggested a low efficiency of energy transfer.

The differences in the visible region between the two excitation spectra can be seen in more detail in the spectrum of Fig. 5, which was obtained by subtraction of the spectra after normalization in the BChl *a* region near 600 nm. The difference spectrum shows bands at 458, 489 and 521 nm which are presumably due to a carotenoid; the bands at 670, 438 and 420 nm are probably due to a (dihydro)porphyrin.

The excitation spectra of the reaction center pigment-protein complex, which contains about 35 BChl *a* molecules per reactions center (Fig. 6), were very similar to those of the pigment-protein complex. In particular, it is interesting to note that the excitation spectrum for the emission at 828 nm was again very similar to that of the soluble BChl *a* protein, that is removed upon preparation of the reaction center pigment-protein complex [1]. This indicates that the reaction center pigment-protein complex still contains an antenna protein that is very similar to the soluble BChl *a* protein, as was earlier concluded from the linear dichroism spectra [5] and from peptide analysis [17]. Also for this BChl protein, the efficiency of energy transfer to the reaction center component emitting at 838 nm is low.

We also tested the relative activities at various wavelengths of illumination in bringing about the oxidation of the primary electron donor P-840. Since the actinic light was filtered by interference filters, the spectral resolution was low. The rates of photooxidation of P-840 were measured at room temperature. The actinic effects of light of about 600 and about 670 nm were found to be roughly the same. This indicates that the action spectrum for P-840 oxidation is probably similar to that for excitation of the 838-nm emission, which supports the hypothesis that the emission originates from the reaction center complex.

The origin of the long-wave emission

The experiments reported above clearly indicate that the fluorescence at 838 nm is emitted by BChl *a* that is fairly closely associated with the reaction center. The relatively high intensity of emission in the pigment-protein and reaction center pigment-protein complexes as compared to Complex I [6,8] might suggest that the intensity of emission is related to the integrity of the reaction center complex, and that the high intensity in the isolated pigment-protein complex is due to impaired energy or electron transfer (see also Ref. 2). This then might also explain the absence of long-wave emission in intact cells [6,9,10,13], but it should be kept in mind that for intact cells the conditions of

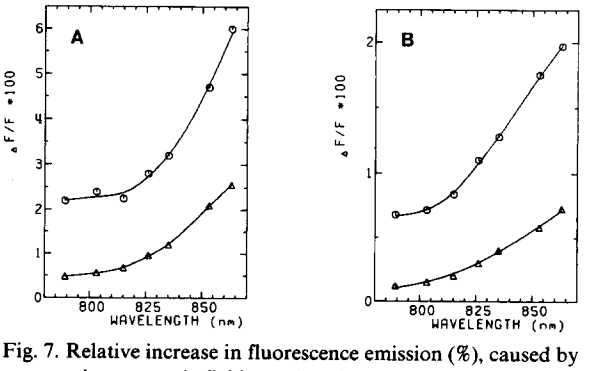

Fig. 7. Relative increase in fluorescence emission (%), caused by a saturating magnetic field as a function of wavelength at room temperature upon excitation at 679 nm (○) or 606 nm (△). (A) Reaction center pigment-protein complex; (B) pigment-protein complex in the presence of 5 mM dithionite.

excitation are quite different due to the large antenna.

In order to check if the long-wave emission consists of delayed fluorescence caused by charge recombination, we examined the effect of a magnetic field on the intensity of emission. It has been shown that such effects occur in the fluorescence emission of chromatophores of purple bacteria and in the fast emission components of delayed light emission in reaction center preparations. In all these cases a stimulation of the emission was observed, which was explained by an increase, in a magnetic field, of the lifetime of the singlet state of the radical pair [18].

Fig. 7 shows the effects of a magnetic field on the emission by the reaction center pigment-protein and pigment-protein complexes measured at various wavelengths of emission and excitation at room temperature. A stimulation of emission was indeed observed, which occurred mainly in the long-wave component. The effect was stronger upon excitation at 679 than at 606 nm. With the pigment-protein complex, a marked stimulation was only observed in the presence of dithionite (Fig. 7B), the addition of which approximately doubled the fluorescence yield. In the absence of dithionite, the stimulation was only 0.4% at 835 nm upon excitation at 679 nm. For both preparations, the stimulation was half-saturated at 0.08 T (data not shown). Since the emission was analyzed by means of interference filters, emission spectra of sufficient resolution of the increase of fluorescence were not obtained in these experiments. Except for amplitude, we found no evidence for a difference in the spectral distribution upon excitation at 606 or 679 nm.

The stimulation by a magnetic field was dependent on temperature. Cooling from 294 to 273 K diminished the effect by a factor of 2.6 for both preparations; it was reduced to about one-fourth at 255 K and no effect at all was observed at temperatures below about 150 K.

Discussion

The results presented here demonstrate that for the isolated pigment-protein complexes the action spectra at low temperature for BChl *a* emission at 828 and 838 nm are clearly different. They indicate that the 828-nm emission comes from an antenna BChl *a* protein complex that exists in two forms: in a soluble form which is attached to the pigment-protein complex, but released upon treatment with guanidine hydrochloride [1], and in a bound form which is part of the reaction center pigment-protein complex. These forms are spectroscopically very similar as indicated by the excitation spectra, which, for both complexes are quite similar to the absorption and excitation spectra of the isolated soluble BChl *a* protein. The efficiency of energy transfer from the antenna complexes to the reaction center complex is low. At 4 K this efficiency is less than 30% in the pigment-protein complex; emission spectra obtained with different wavelengths of exciting light [6,8] indicate that this applies also to room temperature. The low efficiency of energy transfer is possibly due to the isolation procedure; in intact cells energy transfer from the antenna complex to the reaction center is assumed to be efficient, since the antenna complex is supposed to act as an intermediate in energy transfer from the chlorosome to the reaction center [19,20]. It is interesting to note, however, that the mutual orientations of the antenna and reaction center complexes are not significantly altered during the isolation [5].

The emission at 838 nm probably comes from BChl *a* associated with the reaction center. The effects of a magnetic field indicate that at room temperature at least part of the emission is delayed fluorescence brought about by a reversal of the primary charge separation. The recombination is probably favored by damage to the acceptor chain caused by the isolation procedure [2]. For the pigment-protein complex the stimulation was much stronger in the presence of dithionite, when the secondary acceptor X_2 was probably kept in the reduced form by the excitation light [3]. However, the absence of a magnetic field effect below 150 K suggested that at low temperature all of the emission is prompt fluorescence. Its relatively high intensity may be explained by a decrease in the rate of energy transfer from the long-wave emitting BChl *a* to other pigments and perhaps also to the reaction center; this energy transfer may be impaired in the isolated complexes as compared to more intact systems.

In addition to bands of BChl *a*, the action

spectrum for 838-nm fluorescence shows contributions by carotenoid and by at least one other pigment, with bands at 670, 438 and possibly near 420 nm. The data presented in the preceding paper [7] shows that two different pigments absorbing near 670 nm are present in the pigment-protein and reaction center pigment-protein complexes: BPh *c* and the unidentified porphyrin (P-665). The shape of the band near 670 nm in the excitation spectra, as can be most clearly seen in the spectrum of Fig. 5, indicates that energy transfer from only one of these pigments contributes significantly to the emission at 838 nm. The prominent band at 438 nm in the excitation spectrum indicates that this pigment is P-665, rather than BPh *c* if we compare the excitation spectra of the isolated pigments [7]. The significance of the presence of both pigments in the reaction center complex remains obscure, as discussed in the preceding paper [7]. The emission at 674 nm is probably due to BPh *c* as indicated by the bands at 480 nm, 515 and 550 nm in the excitation spectrum, but the relative amplitude of these bands was small and variable, and part of the emission is probably due to strongly fluorescing degradation products [8].

The carotenoid that can be observed in the excitation spectrum of the 838 nm emission, with bands at 458, 489 and 521 nm, is spectroscopically different from the bulk carotenoid visible at 461, 493 and 528 nm in the absorption spectrum. This suggests that the carotenoid may represent a small pool that transfers its energy fairly efficiently to the reaction center. The significance of this carotenoid is not known; there is no evidence [2] that it protects against photooxidative damage as do carotenoids in purple photosynthetic bacteria [21].

Acknowledgements

Thanks are due to Mrs. L.M. Blom and to R.H. Voogd for preparation of the pigment-protein complexes, to A.H.M. de Wit for culturing the bacteria and to Mrs. K.M. van der Veek-Horsley for measurements of P-840 oxidation. The investigation was supported by the Netherlands Foundation for Chemical Research (SON) and for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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