Biochimica et Biophysica Acta, 592 (1980) 53—64

BBA 47899

ENERGY TRANSFER AND BACTERIOCHLOROPHYLL FLUORESCENCE IN PURPLE BACTERIA AT LOW TEMPERATURE

C.P. RIJGERSBERG, R. VAN GRONDELLE and J. AMESZ

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

(Received January 17th, 1980)

Key words: Energy transfer; Bacteriochlorophyll fluorescence; Fluorescence yield; Quantum yield; Photosynthetic bacterium; (Purple bacteria)

Summary

Emission spectra of bacteriochlorophyll *a* fluorescence and absorption spectra of various purple bacteria were measured at temperatures between 295 and 4 K. For *Rhodospirillum rubrum* the relative yield of photochemistry was measured in the same temperature region.

In agreement with earlier results, sharpening and shifts of absorption bands were observed upon cooling to 77 K. Below 77 K further sharpening occurred. In all species an absorption band was observed at 751—757 nm. The position of this band and its amplitude relative to the concentration of reaction centers indicate that this band is due to reaction center bacteriopheophytin. The main infrared absorption band of *Rhodopseudomonas sphaeroides* strain R26 is resolved in two bands at low temperature, which may suggest that there are two pigment-protein complexes in this species.

Emission bands, like the absorption bands, shifted and sharpened upon cooling. The fluorescence yield remained constant or even decreased in some species between room temperature and 120 K, but showed an increase below 120 K. This increase was most pronounced in species, such as *R. rubrum*, which showed single banded emission spectra. In *Chromatium vinosum* three (835, 893 and 934 nm) and in *Rps. sphaeroides* two (888 and 909 nm) emission bands were observed at low temperature. The temperature dependence of the amplitudes of the short wavelength bands indicated the absence of a thermal equilibrium for the excitation energy distribution in *C. vinosum* and *Rps. sphaeroides*. In all species the increase in the yield was larger when all reaction

---

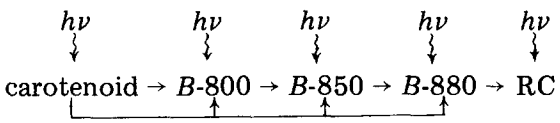
Abbreviations: BChl, bacteriochlorophyll; *P*-870, *P*-800, reaction center bacteriochlorophylls; *B*-800, *B*-850, *B*-855, *B*-870, *B*-880, *B*-890, antenna bacteriochlorophylls with maximum absorption near the wavelength indicated.

centers were photochemically active than when the reaction centers were closed.

In *R. rubrum* the increase in the fluorescence yield was accompanied by a decrease of the quantum yield of charge separation upon excitation of the antenna but not of the reaction center chlorophyll. Calculation of the Förster resonance integral at various temperatures indicated that the increase in fluorescence yield and the decrease in the yield of photochemistry may be due to a decrease in the rate of energy transfer between antenna bacteriochlorophyll molecules. The energy transfer from carotenoids to bacteriochlorophyll was independent of the temperature in all species examined. The results are discussed in terms of existing models for energy transfer in the antenna pigment system.

Introduction

In purple bacteria most of the energy used for photochemistry is not directly absorbed by the reaction center, but by the so-called antenna pigments, BChl *a* and carotenoids. Within the BChl antenna the excitation transfer takes place from BChl *a* absorbing at shorter to BChl *a* absorbing at longer wavelength. For example in *Rps. sphaeroides*, the energy sequence can be schematized in the following way [1,2]:



In this scheme *B*-800, *B*-850 and *B*-880 represent pools of antenna bacteriochlorophyll molecules having their absorption maximum near 800, 850 and 880 nm, respectively [1,3,4]. In some species additional pigment pools are present (e.g. *C. vinosum*, which contains in addition *B*-820), in other species some of these pigment pools are absent (e.g. *R. rubrum*, which contains only *B*-880). The scheme given above would suggest that the carotenoids which are present may transfer their energy to all of the bacteriochlorophyll types. However, since at least part of the carotenoids are incorporated in so-called bacteriochlorophyll-carotenoid-pigment protein complexes, it is very likely that for these carotenoids energy transfer occurs only to the associated bacteriochlorophyll.

Once the excitation energy has reached one of the long-wave absorption bacteriochlorophyll molecules, the energy is transferred mainly among these molecules until the reaction center bacteriochlorophyll dimer (*P*-870) becomes excited and primary photochemistry may be initiated [5,6]. To describe the energy transfer between BChl *a* molecules within the pools and from one pool to another the Förster mechanism can be used. For a donor (D) and an acceptor (A) separated by a distance R the rate of energy transfer, k_{DA}, is given by [1,7,8]:

$$k_{DA} = \frac{\phi_D}{\tau} \frac{1}{R^6} \left[\frac{3}{4\pi} \int_0^\infty \lambda^4 F_D(\omega)\, \sigma_A(\omega)\, d\omega \right] \quad (1)$$

where ϕ_D is the fluorescence yield of the donor in the absence of an acceptor, τ is the lifetime in the absence of an acceptor, $\lambda\!\!\!^{-} = \lambda/2\pi n$, where λ is the wavelength and n the index of refraction of the medium, $F_D(\omega)$ is the normalized fluorescence emission spectrum of the donor on an angular frequency scale, $\sigma_A(\omega)$ is the absorption cross section of the acceptor. The expression between the brackets is often called the overlap or resonance integral. The probability that the absorbed energy is finally transferred to the trap and is used for photochemistry is high (>90%) [9,10], but there exists a small proability that the excitation is lost by fluorescence, internal conversion, or intersystem crossing.

In the experiments described in this paper the absorption spectra and fluorescence emission spectra have been measured as a function of the temperature down to 4 K in a number of photosynthetic bacteria, some with only one absorption and emission band, others with several pools of antenna bacteriochlorophylls present. For the species with a single antenna pool it was possible to calculate the overlap integral (Eqn. 1), a measure for the rate of energy transfer, as a function of the temperature. Changes in the value of the overlap integral with temperature due to band sharpening were obtained, and compared with the variations in fluorescence yield and yield of photochemistry as a function of temperature in the same species. The results indicate that temperature lowering results in a decrease in the rates of energy transfer, which in turn causes a decrease of the probability of trapping of excitation energy by the reaction center and a concomitant increase in the yield of fluorescence of antenna bacteriochlorophyll.

Materials and Methods

Cells of *Rhodospirillum rubrum* strain S1, *Rhodospirillum rubrum* mutant strain FR1 VI, kindly provided by Dr. J. Oelze, *Rhodopseudomonas sphaeroides* strain 2.4.1 and *Rhodopseudomonas sphaeroides* mutant strain R26 were grown in a medium after Cohen-Bazire et al. [11], supplied with yeast extract and peptone. *Chromatium vinosum* strain D was cultured in a medium after Hendley [12]. The cultures were gassed with N_2 during growth. The cells were harvested by centrifugation and resuspended in fresh growth medium. Just before the experiments, sucrose and glycerol, final concentrations 1 M and 70% v/v, were added to obtain clear samples upon cooling.

The single beam spectrophotometer and the cryostat used were described before [13,14]. For the measurements of fluorescence spectra the monochromator was supplemented by a Schott KV 550 and a Schott RG 715 filter to absorb scattered excitation light; the bandwidth was set at 1.6 nm. The photomultiplier used for fluorescence measurements (S1 type) was mounted in a thermostated liquid nitrogen-cooled housing and operated at 170 K.

The excitation light was selected by an absorption filter (Schott BG 18) combined with a Balzers K3 or K5 broad-band interference filter, to excite preferentially carotenoid and bacteriochlorophyll, respectively. The intensity of the excitation light was attenuated by Schott neutral density filters. The absorbance of the samples used for fluorescence measurements was always less than 0.2 in the long wavelength peak. The fluorescence was detected at the illuminated surface of the vessel. The excitation light for the absorption dif-

ference measurements was provided by a xenon flash in combination with absorption (Schott RG 780) and interference (Schott AL 803 or 821 nm) filters. All samples were contained in a perpex (lucite) vessel of 1 mm thickness. A computer program was used to plot the fluorescence emission spectra (after correction for the wavelength dependent sensitivity of the apparatus) and the absorbance spectra and to calculate the integrated fluorescence and the overlap integrals. The emission spectra are plotted in arbitrary units proportional to Watts per wavelength interval. The second derivatives of the absorbance spectra were determined according to the method given in Ref. 15.

Results

Absorption spectra

Fig. 1A shows absorption spectra of *R. rubrum* at room temperature, 80 K and 4 K. The spectrum shows only two bands in the near-infrared region at room temperature. Both bands, located at 803 nm and at 883 nm respectively, are due to BChl *a*. The bacteriochlorophyll absorbing at 803 nm is due to the two *P*-800 bacteriochlorophyll molecules incorporated in the reaction center complex [5,16]. Upon cooling to 80 K the 803 band and the 883 band are sharpened and the 883 band shifts to 897 nm [17]. At this temperature we also observe a small absorption maximum at 752 nm which is due to reaction center bacteriopheophytin *a* [16] as will be discussed below. Further cooling to 4 K results only in an increased narrowing of the absorption bands. The spectrum of a blue-green mutant of *R. rubrum* (FR1 VI) [18] resembled that of the wild type [18], but the peak positions were somewhat shifted (Table I) and the absorption band at the longest wavelength was broader.

Fig. 1B shows the spectra of *Rps. sphaeroides*. At room temperature the bacteriochlorophyll maxima are located at 801 and 852 nm. The latter band shows a shoulder at the long wavelength side. Upon cooling the narrowing of the bacteriochlorophyll peaks is observed. The long wavelength absorption peak shifts to 855 nm; the shoulder already present at room temperature

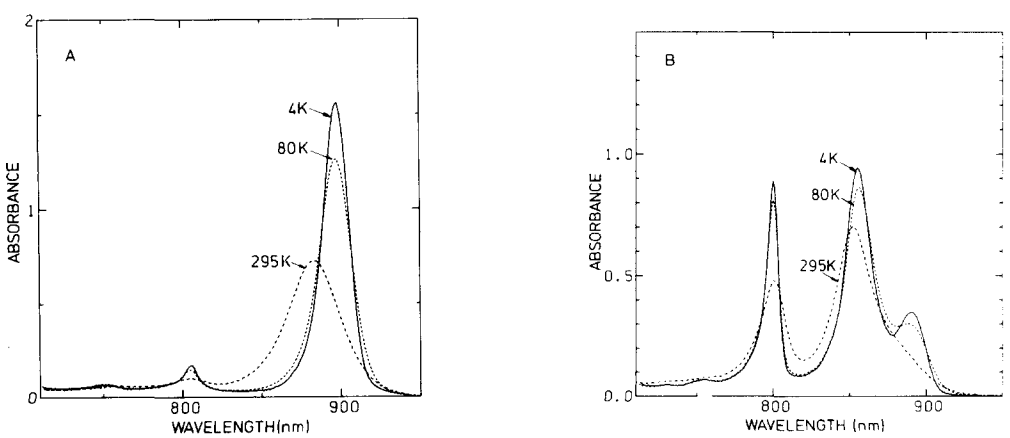


Fig. 1. Absorption spectra of whole cells of *R. rubrum* strain S1 (A) and of *Rps. sphaeroides* strain 2.4.1 (B) at different temperatures.

develops into a clearly separated band. At 4 K the maxima are located at 800, 855 and 892 nm. The band at 754 nm is probably due to bacteriopheophytin. In the R26 mutant of *Rps. sphaeroides*, which lacks carotenoids and *B*-800 and *B*-850 [19], we observed an asymmetric band at about 870 nm in the absorption spectrum below 100 K (Fig. 2). Analysis of the absorption spectrum by means of the second derivative spectrum showed that this peak was a composite of two bands located at 766 and 888 nm at 4 K. The spectrum of *C. vinosum* showed the well-known five bacteriochlorophyll peaks [17] in addition to the bacteriopheophytin band (756 nm) at low temperature. The peak positions are given in Table I and are similar to those obtained by others at 77 K [4,17,20].

Fluorescence emission spectra

Fig. 3A shows emission spectra of *R. rubrum* recorded at various temperatures with light of low intensity such that most traps remained open during illumination. Compared to the spectrum measured at room temperature the spectrum at 80 K is shifted towards longer wavelength. Between 295 and 80 K the fluorescence yield did not change appreciably, but below 80 K the amplitude of the fluorescence increased considerably while the bandwidth decreased. In all species examined we found that the fluorescence yield depended on the state of the photochemical trap. For conditions with photochemically inactive traps (when *P*-870 is in the oxidized state), the increase of the so-called maximum yield of fluorescence (ϕ_{max}) as a function of the temperature was smaller than the yield measured with open traps (ϕ_o). In the species with a single-banded emission spectrum the shape of the spectrum was independent of the light intensity. Fig. 3B shows the value of the integrated emission as a function of temperature recorded at low intensity (ϕ_o) and at an intensity which gave a yield close to ϕ_{max}, as was checked by varying the intensity of excitation. In some cultures the increase upon cooling was stronger than shown

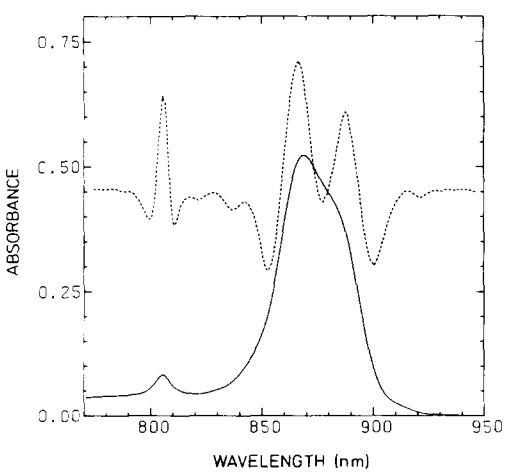


Fig. 2. Absorption spectrum of *Rps. sphaeroides* R26 at 4 K (solid line). The dashed curve shows the second derivative spectrum (inverted) on an arbitrary scale.

TABLE I

PEAKS AND SHOULDERS (s) OBSERVED IN ABSORBANCE AND EMISSION SPECTRA OF VARIOUS SPECIES AT ROOM TEMPERATURE AND AT 4 K

The positions of the maxima in the absorption spectra were determined from the second derivative spectra.

Species	Absorbance (nm)		Fluorescence (nm)	
	293 K	4 K	293 K	4 K
R. rubrum strain S1		751		
	804	805		
	883	897	900	918
R. rubrum FR1 VI		752		
	805	805		
	875	886	892	912
Rps. sphaeroides strain 2.4.1		755		
	801	800		
	852	855	866(s)	888(s)
	890	893	895	909
Rps. sphaeroides R-26		757		
	805	805		
	858	866		
	885(s)	887	894	909
C. vinosum		756		
	797(s)	795		
	808	808		
	822(s)	822		835(s)
	850(s)	860	861(s)	893
	892	910	913	937

in Fig. 3 (e.g. Fig. 6). It should be noted that the high intensities used to obtain ϕ_{max} caused heating of the sample by some degrees, especially at low temperature. This, however, presumably did not introduce a gross error in the results. The mutant FR1 VI of *R. rubrum* also showed a single emission band,

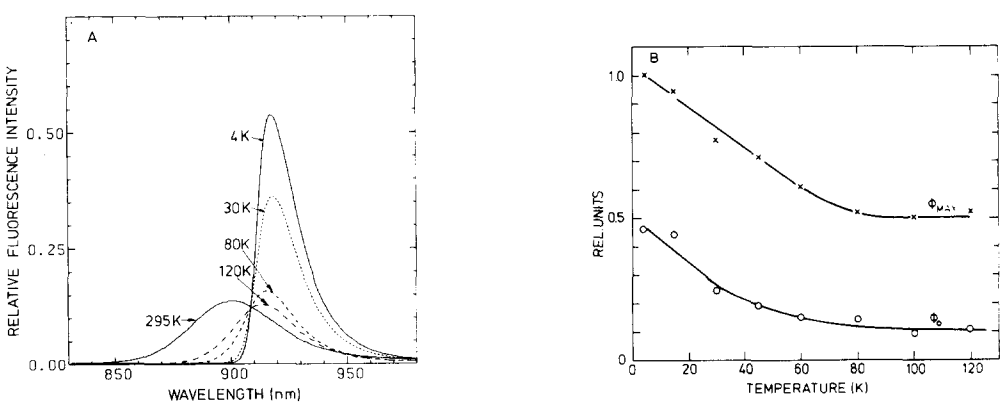


Fig. 3. A. Fluorescence emission spectra of *R. rubrum* S1 at five different temperatures; excitation with a band of 575—610 nm; intensity 1 mW/cm^2. B. Temperature dependence of ϕ_0 and ϕ_{max} (see text), recorded with actinic intensities of 0.09 and 50 mW/cm^2, respectively.

located at shorter wavelength and broadened compared to the wild type, in analogy to the absorption spectrum (Table I). The yield of ϕ_o and ϕ_{max} showed a similar dependence on the temperature as was observed for the wild type, with a 3.5-fold increase in ϕ_o between 80 K and 4 K.

Emission spectra of *Rps. sphaeroides* (Fig. 4A) showed two overlapping emission bands at all temperatures. The bands are most clearly discerned at 4 K under ϕ_o conditions. Between 100 and 4 K the variable fluorescence yield (ϕ_v, the difference between ϕ_{max} and ϕ_o) contributed only to the long wavelength band, located at 909 nm (Fig. 4B). A short wavelength band has been earlier observed at room temperature [21] but not at 77 K [20], probably due to the high intensity of the excitation light used. At temperatures below 80 K the ratio of the emission intensities at 890 and 906 nm remained roughly the same. The distribution of the excited pigment molecules among the pools of BChl *a* emitting at these wavelengths is thus clearly not in agreement with a thermal equilibrium [22].

The spectra of *Rps. sphaeroides* strain R26 showed only one band under all conditions. This band was shifted by 2—3 nm toward longer wavelength and was much broader (30 nm as compared to 21 nm) than the main band of the wild type. *C. vinosum* showed two bands at 293 K (Table I). The spectrum at 4 K showed bands at 893 and 937 nm, and in addition to these a weak shoulder (Fig. 5) at 835 nm at low temperatures. Like with *Rps. sphaeroides*, the short wave band was only present in the low temperature spectrum of ϕ_o, but not in the ϕ_v spectrum; again the ratio of the two bands was approximately constant between 80 K and 4 K. The increase in fluorescence yield upon cooling was larger in the species with a single emission band than in species with multi-banded emission spectra.

Energy transfer between carotenoids and BChl a

By comparing the emission spectra recorded at various temperatures upon

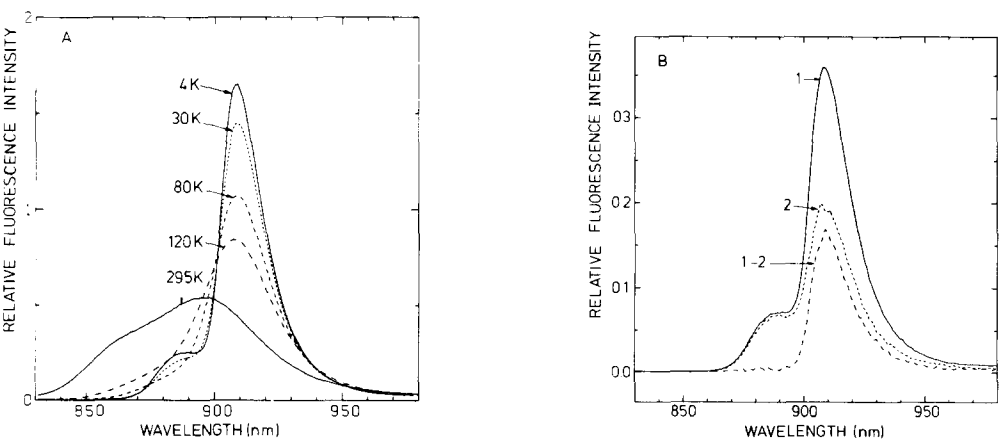


Fig. 4. A. Fluorescence emission spectra recorded at high light intensity of *Rps. sphaeroides* 2.4.1 at five difference temperatures. Excitation with a band of 575—610 nm; intensity 3 mW/cm^2. B. Emission spectra recorded with high (1) and low (2) intensity of excitation light (480—530 nm; 600 and 60 μW/cm^2, respectively), and spectrum of the variable fluorescence (1 — 2) at 4 K. The emission intensities were divided by the incident intensity and plotted on the same arbitrary scale.

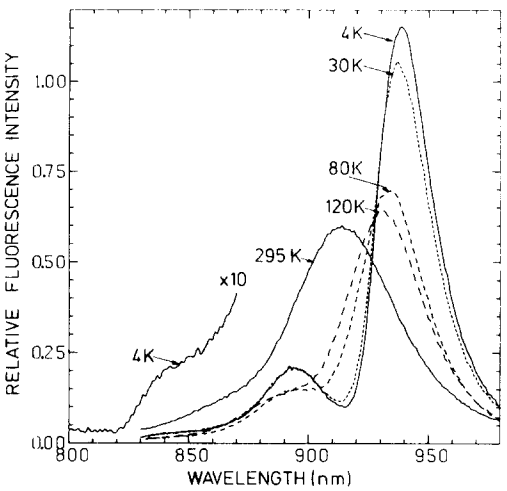


Fig. 5. Fluorescence emission spectrum of *C. vinosum* at five different temperatures. Part of the spectrum at 4 K is drawn on a 10-fold expanded scale. Excitation with a band of 480—530 nm; intensity 0.6 mW/cm^2.

excitation with light preferably absorbed by carotenoids or by bacteriochlorophyll, the effect of cooling on the efficiency of energy transfer between carotenoids and bacteriochlorophyll was determined. For all species containing carotenoids we found that the dependence of the total emission on the temperature was the same upon excitation at 480—530 nm and at 575—610 nm. This indicates that the transfer efficiency from carotenoid to bacteriochlorophyll is temperature independent. Within an accuracy of 5—10%, the shape of the emission spectra, for all species examined, was not dependent on the excitation wavelength, both in ϕ_o and ϕ_{max} conditions.

Overlap integral calculations

According to the theory of resonance energy transfer formulated by Förster [7], the rate of energy transfer is proportional to the overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor molecules (see Eqn. 1). For *R. rubrum* the emission and absorption spectra of the antenna bacteriochlorophyll are single banded. If we assume that these spectra represent the absorption and emission spectra of the individual bacteriochlorophyll molecules in the antenna, the resonance integral can be calculated as a function of temperature. Fig. 6 shows the result of such a calculation. It can be seen that the variation with temperature approximately mirrors that observed for the fluorescence yield. Essentially the same results were obtained with *R. rubrum* FR1 VI. These results strongly suggest that the increase in the fluorescence is due to a decrease in the rate of energy transfer between the individual bacteriochlorophyll molecules.

Temperature dependence of the yield of charge separation

In order to determine if the decrease in the energy transfer rates calculated by the overlap integral is also reflected in a lowering of the apparent rate of

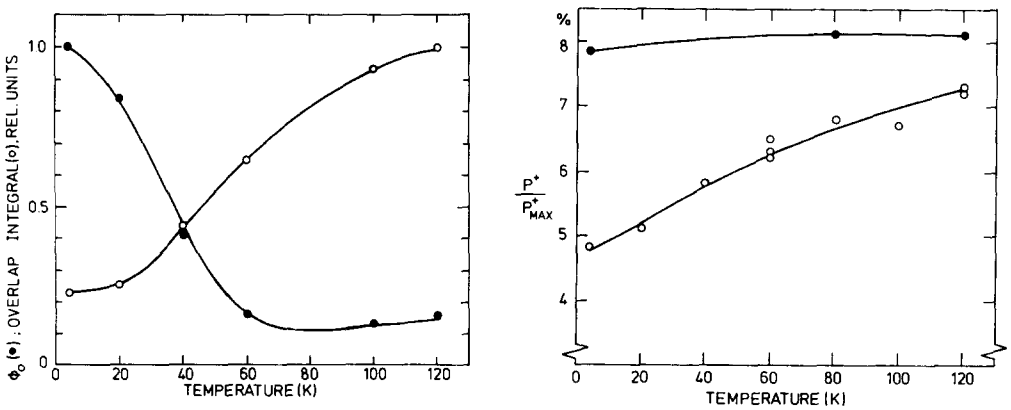


Fig. 6. Overlap integral (see Eqn. 1) (○———○) and the relative fluorescence yield (●———●) for *R. rubrum* S1 as a function of temperature.

Fig. 7. Relative yield of photochemistry in *R. rubrum* S1 induced by non-saturating xenon flashes (7 μs) as a function of temperature. The amount of absorbed light was equal at all temperatures. The amount of P^+-870 formed was calculated relative to that formed in a saturating flash transmitted by a RG 780 filter. ○———○, Illumination 821 nm; ●———●, illumination 803 nm.

photochemistry, we measured the extent of bleaching at 610 nm, due to oxidation of *P*-870, induced by non-saturating flashes of various intensities. For this experiment *R. rubrum* was used because the increase in the fluorescence yield is most pronounced in this species. At 120 K, the yield of *P*-870 oxidation was, within the limits of accuracy of the experiment, the same as at room temperature and therefore probably higher than 0.9 electron transfer per light quantum absorbed [9,10]. Between 120 K and 4 K we observed a considerable decrease (30—40%) of the quantum yield upon illumination with a band around 821 nm, or with light transmitted by a colored glass filter absorbing below 780 nm. In both cases the actinic light is mainly absorbed by antenna bacteriochlorophyll. However, upon illumination at 803 nm, which wavelength is mainly absorbed by the reaction center pigment *P*-800, we observed a decrease of only 5% upon cooling from 120 K to 4 K (Fig. 7). Since at least part of this decrease must be due to 'background' absorption at this wavelength by antenna pigments, it may be concluded that the photochemical activity of the reaction center is essentially not affected by cooling, in agreement with results obtained with isolated reaction centers of *Rps. sphaeroides* [23]. The observed decrease in efficiency thus can be ascribed to a lowering of the efficiency of energy transfer of light absorbed by the antenna bacteriochlorophyll to the reaction center.

Discussion

Upon cooling to 80 K, the absorption spectra of all species showed a sharpening of the near-infrared bands and a shift towards longer wavelength of the long-wave bands, in agreement with earlier observations [17]. Further cooling to 4 K resulted in an increased narrowing. In all species we observed a band at 751—757 nm which is probably due to bacteriopheophytin. Com-

parison of the amplitude of this band relative to that of the reaction center bacteriochlorophyll at 805 nm in *R. rubrum* and to the extent of bleaching of *P*-870 in saturating light (not shown) showed that within the accuracy of the measurement, the amount of bacteriopheophytin corresponds to that incorporated in the reaction centers. The amplitude of the band in other species is of similar magnitude in relation to *P*-800 or *P*-870. This indicates strongly that there is no bacteriopheophytin present in the intact cell other than that belonging to the reaction center. In *Rps. sphaeroides* mutant strain R26 we observed a clear splitting in the near-infrared band at low temperature with two components at 866 and 887 nm. Since Sauer and Austin [24] observed only one band in the long-wave region of the spectrum of the *B*-855 pigment-protein complex recorded at low temperature, this may suggest that R26 contains a *B*-870 complex in addition to the *B*-855 complex.

Like the absorption spectra, the fluorescence emission spectra showed shifts and narrowing of the bands upon cooling. The emission spectra of *R. rubrum* and *Rps. sphaeroides* R26 have a single maximum; those of *Rps. sphaeroides* 2.4.1 and of *C. vinosum* show two and three bands, respectively, at low temperature. The relative amplitudes of these bands were similar upon carotenoid and bacteriochlorophyll excitation. At first sight this would seem to suggest that the ratio of carotenoid to bacteriochlorophyll is the same in the different BChl-protein complexes. This would be at variance with the composition of isolated BChl-protein complexes as e.g. reported by Cogdell and coworkers [4,25,26]. However, it should be kept in mind that, as judged from the absorption spectra, the amount of excitation absorbed by the *B*-800-*B*-850 complex exceeds that absorbed by the *B*-890 complex quite considerably with either wavelength of excitation, so that the expected effect should be small, even if the efficiency of energy transfer between carotenoid and bacteriochlorophyll is the same in both complexes. More accurate and extensive experiments would be needed to resolve this point.

The temperature independence of energy transfer between carotenoid and bacteriochlorophyll observed for all species tested might be explained by the existence of two pools of carotenoid, with transfer efficiencies of close to 100 and 0%, respectively. The first one might represent carotenoid bound in the BChl-carotenoid complexes [4,25,26], the second one carotenoid occurring in carotenoid-protein complexes devoid of bacteriochlorophyll [27]. However, the triplet quenching observed by Rademaker et al. [28] suggests that, at least in *R. rubrum*, essentially all carotenoid is closely associated with antenna bacteriochlorophyll. Another explanation might be that the mechanism of energy transfer between carotenoid and bacteriochlorophyll, which is probably not of the Förster dipole-dipole type of interaction [29], but occurs by a short range transfer mechanism, is less temperature sensitive.

The temperature dependence of the emission bands of *Rps. sphaeroides* 2.4.1 and of *C. vinosum* shows that the excitation energy is not distributed among the bacteriochlorophyll pools according to a thermal equilibrium, where the excitation density in each pool is governed only by the energy difference between the two pools, the temperature and a trapping rate [22]. This might be due to a topology of the antenna system with relatively large areas of the *B*-800-*B*-850 complexes, as proposed by Monger and Parson [30]. At very

low temperatures excitation energy arriving in *B*-850 then would take a relatively long time to be transferred to *B*-870. During that time the energy may be lost as *B*-850 fluorescence. Transfer to *B*-870 is irreversible.

We found that for *R. rubrum* S1 at 120 K, the shape and location of the emission and absorption spectra were in approximate agreement with the Stepanov equation [31] applied for 135—140 K. At 4 K a temperature of 35—45 K had to be used and only an approximate fit could be obtained. In a similar way, the short wave emission band in *Rps. sphaeroides* at 4 K (888 nm) corresponded to the absorption maximum at 855 nm if a temperature of about 35 K was used. The latter calculation was based on the estimated bandwidth and on the assumption that the bands were of Gaussian shape (see Ref. 32).

Our results strongly suggest that the increase in the yield of bacteriochlorophyll fluorescence upon cooling can be correlated to the decrease of the rate of energy transfer, calculated from the overlap integral in the Förster equation. This can be explained by the following reasoning: the decrease in the energy transfer results in a decreased number of visited sites during the lifetime of the excitation which in turn decreases the probability that the excited bacteriochlorophyll loses the excitation to reaction centers or to quenchers in the bulk chlorophyll. This argument was supported by the experiments where the apparent yield of photochemistry upon excitation of antenna bacteriochlorophyll was determined. Thus the transfer rate among the long-wavelength bacteriochlorophylls is at 4 K not much higher than all the other rate constants involved in the primary processes of transfer and trapping of excitation energy. This implies that the average transfer time necessary for an excitation to reach the reaction center determines the quantum yield of charge separation. It thus appears that the model for energy transfer in the photosynthetic system discussed by Duysens [33] does not apply at low temperature (e.g. in *R. rubrum* below 60 K) because the fluorescence is a function of the transfer rate under these conditions. A comparison of the results with model calculations suggested that even at room temperature the rate of energy transfer in the antenna pigments is of the same order of magnitude as the rate of excitation trapping in the reaction center (van der Wal, H.N., personal communication). The increase in the fluorescence yield under ϕ_{max} conditions upon cooling indicates that there are quenchers in this state too. This quenching may be due to closed reaction centers [33] or special pigments in the antenna [34]. The latter situation seemed to apply to a *B*-800-*B*-850 pigment-protein complex, isolated from a reaction center less mutant of *Rps. capsulata*, which complex also showed an increase in the fluorescence yield concomitant with a decrease of the overlap integral (Rijgersberg, C.P., van Grondelle, R. and Feick, R., unpublished observations).

Acknowledgements

This investigation was supported by the Netherlands Foundations for Chemical Research (SON) and for Biophysics, financed by the Organization for the Advancement of Pure Research (ZWO) and by the EEC solar energy programme (contract No. 027-76-ESN). The authors are indebted to Mr. D. Los and Ing. J.H.M. van de Water for writing the computer programs, to Mr. A.H.M.

de Wit for culturing the bacteria and to Mr. F.A.L.G. Janssen for assisting with some of the experiments.

References

1 Duysens, L.N.M. (1952) Transfer of Excitation Energy in Photosynthesis, Thesis, Utrecht
2 Zankel, K.L. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 341—347, Plenum Publishing Corporation, New York
3 Amesz, J. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 333—340, Plenum Publishing Corporation, New York
4 Thornber, J.P., Trosper, T.L. and Strouse, C.E. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 133—160, Plenum Publishing Corporation, New York
5 Parson, W.W. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 416, 105—149
6 Van Grondelle, R. (1978) A Study on Primary and Cytochrome Reactions in Bacterial Photosynthesis, Thesis, Leiden
7 Förster, Th. (1948) Ann. Physik 2, 55—75
8 Duysens, L.N.M. (1964) Progress in Biophysics 14, 1—104
9 Olson, J.M. (1962) Science 135, 101—102
10 Vredenberg, W.J. and Duysens, L.N.M. (1964) Biochim. Biophys. Acta 79, 456—463
11 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell Comp. Physiol. 49, 25—68
12 Hendley, D.D. (1955) J. Bacteriol. 70, 625—634
13 Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473—482
14 Rijgersberg, C.P. and Amesz, J. (1978) Biochim. Biophys. Acta 502, 152—160
15 Butler, W.L. and Hopkins, D.W. (1970) Photochem. Photobiol. 12, 439—450
16 Prince, R.C. and Thornber, J.P. (1977) FEBS Lett. 81, 233—237
17 Vredenberg, W.J. and Amesz, J. (1966) Biochim. Biophys. Acta 126, 244—253
18 Oelze, J. and Golecki, J.R. (1975) Arch. Microbiol. 102, 59—64
19 Clayton, R.K. (1963) Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 495—500, Antioch Press, Yellow Springs, OH
20 Goedheer, J.C. (1972) Biochim. Biophys. Acta 275, 169—176
21 Goedheer, J.C. and van der Tuin, A.K. (1967) Biochim. Biophys. Acta 143, 399—407
22 Zankel, K.L. and Clayton, R.K. (1969) Photochem. Photobiol. 9, 7—15
23 Clayton, R.K. and Yamamoto, T. (1976) Photochem. Photobiol. 24, 67—70
24 Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011—2019
25 Cogdell, R.J. and Thornber, J.P. (1979) in Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Foundation Symposium 61 (New Series), pp. 61—79, Excerpta Medica, Amsterdam
26 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409—416
27 Schwenker, U. and Gingras, G. (1973) Biochem. Biophys. Res. Commun. 51, 94—99
28 Rademaker, H., Hoff, A.J., van Grondelle, R. and Duysens, L.N.M. (1980) Biochim. Biophys. Acta, 592, in the press
29 Song, P.S. and Moore, T.A. (1974) Photochem. Photobiol. 19, 435—441
30 Monger, T.G. and Parson, W.W. (1977) Biochim. Biophys. Acta 460, 393—407
31 Stepanov, B.I. (1957) Doklady Akad. Nauk. S.S.S.R. 112, 839—841; (1957) Sov. Phys.-Doklady 2, 81—84
32 Kazachenko, L.P. (1965) Optics and Spectroscopy (U.S.S.R.) 18, 397—398; (1965) Optika i Spektrosk. 18, 708—709
33 Duysens, L.N.M. (1979) in Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Foundation Symposium 61 (New Series), pp. 323—340, Excerpta Medica, Amsterdam
34 Van Grondelle, R. and Duysens, L.N.M. (1980) Plant Physiol., in the press