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Energy transfer from long-wavelength absorbing antenna bacteriochlorophylls to the reaction center

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Charge separation was studied in membranes of *Rhodopseudomonas viridis* and *Heliobacterium chlorum* upon flash excitation in the bacteriochlorophyll (BChl) Q_y band. In the first organism the maximum of the Q_y absorption of the antenna BChl is situated at significantly longer wavelength than that of the primary electron donor, whereas in *H. chlorum* the same applies to the long-wavelength absorbing form BChl *g*-808. In both species the photochemical efficiency was independent of the wavelength of excitation throughout the Q_y absorption region, both at 300 K and 6 K. Upon cooling to 6 K the absolute efficiency decreased by 29 ± 4% in *H. chlorum* and by 55 ± 5% in *Rps. viridis*. The fluorescence excitation spectrum of *Rps. viridis* membranes at 6 K showed virtually no contribution by reaction center pigments. This indicates that the efficiency for back transfer of energy from the excited reaction center to the antenna was at most a few percent and may be explained by a strong competition by the much faster rate of charge separation. In the absence of back transfer our results allowed an estimation of the rate constant for energy transfer from neighboring antenna BChls to the reaction center at 6 K, which were found to be (440 ps)$^{-1}$ for *H. chlorum* and (1.3 ns)$^{-1}$ for *Rps. viridis*. It is shown that these rates are not incompatible with the Förster mechanism of energy transfer if it is assumed that the antenna and reaction center bands are essentially homogeneously broadened. Application of the Förster equation yielded an estimated value of 1.8 nm for the distance between the primary electron donor P-798 and BChl *g*-808 in *H. chlorum*.

Introduction

The antenna system of photosynthetic organisms serves to absorb light energy and to transfer this energy to the reaction center. The transfer of energy between antenna (bacterio)chlorophylls is thought to occur by means of induced resonance [1,2]. According to the well-known Förster equation the rate of energy transfer is proportional to, amongst other things, the overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor molecule. Because of the Stokes shift of the fluorescence spectrum with respect to the absorption spectrum, the highest rate of energy transfer will occur when the acceptor molecule absorbs at somewhat longer wavelength than the donor. This means that energy transfer will usually go "down-hill", and that the excitations in the antenna will tend to accumulate at the pigment molecules with the lowest excitation energy. In agreement with this, it has been observed in various antenna systems that most of the fluorescence is emitted from the long-wavelength absorbing chlorophylls or bacteriochlorophylls [1–3].

In a number of cases, the long-wavelength absorbing pigments appear to absorb at significantly longer wavelength than the primary electron donor in the reaction center. A clear example is the BChl *b*-containing purple bacterium *Rhodopseudomonas viridis*, where at 300 K the maximum antenna absorption in the Q_y region is at 1015 nm, whereas the maximum bleaching of the primary electron donor upon oxidation was observed at 985 nm [4]. At liquid helium temperature the maximum antenna absorption is shifted to 1035 nm (see below), whereas the maximum bleaching in the triplet-minus-singlet difference spectrum of the reaction center is situated around 1000 nm [5]. A similar phenomenon is

Abbreviations: BChl, Bacteriochlorophyll; P-798, primary electron donor of *H. chlorum*; P-960, primary electron donor of *Rps. viridis*; PMS, *N*-methylphenazonium methosulphate.

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observed in the recently discovered, BChl g-containing heliobacteria. In these species a considerable fraction of the antenna absorption in the Q_y region at room temperature is located at longer wavelengths than the primary donor, P-798 [3,6]. At low temperature a long wavelength absorbing antenna component, BChl g-808, can be resolved in the absorption spectrum [3,7,8], whereas the primary donor is thought to absorb at 794 nm [8,9].

It thus appears likely that not only in *Rps. viridis*, but also in heliobacteria a considerable gap exists between the energies of the excited states in the antenna and of the primary electron donor. Such a gap may be expected to reduce the efficiency of energy transfer to the reaction center, which seems to contradict the generally held opinion that in the course of evolution energy transfer to the reaction center has been optimized. To help understand the effects of the presence of long-wavelength antenna components, we have performed experiments involving charge separation in membrane fragments of *Rps. viridis* and *Heliobacterium chlorum* upon excitation at selected wavelengths as a function of temperature. It will be shown that for these species the efficiency of charge separation is considerable even at 6 K, and that the efficiency is constant for excitation throughout the Q_y region of antenna absorption at room temperature as well as at low temperature. The results will be interpreted in terms of the Förster mechanism for energy transfer from the antenna to the reaction center.

Materials and Methods

Heliobacterium chlorum was grown and membrane fragments were prepared as described in Ref. 7. They were suspended in a buffer containing 10 mM Tris (pH 8.0) and 10 mM ascorbate. For room-temperature measurements 40 μM PMS was present in the sample. *Rhodopseudomonas viridis* was grown according to Ref. 10. Membrane fragments were suspended in a buffer containing 20 mM Tris (pH 8.0) and mixed with 50% glycerol (v/v) to prevent freezing when stored at -20°C.

Flash induced absorbance difference spectra were measured with a single beam spectrophotometer [6]. For measurements at a fixed wavelength the measuring light was provided by a 250 W tungsten halogen lamp and interference filters were placed behind the light source and in front of the photomultiplier in order to select a suitable wavelength (bandwidth 10 nm). Detection and registration of the signals was performed as described in Ref. 6.

Saturating excitation flashes were provided by the frequency doubled output (532 nm) of a Q-switched Nd-YAG laser (15 ns FWHM). Non-saturating flashes of selected wavelengths were obtained by passing the output of a xenon flashlamp (15 μs FWHM) through a monochromator. Suitable color filters were used to block light of higher order. The sample was contained in a 1 mm cuvette placed at an angle of 45° with both the measuring light and the excitation light. For measurements at low temperature the cuvette was placed in a helium flow cryostat, and the sample contained 66% (v/v) glycerol to prevent crystallization. The temperature of the sample was checked by monitoring the resistance of a silicon diode placed in the sample [11]. Control experiments where the intensity of the measuring light was reduced by a factor of 10 showed that the absorbance changes were not affected by heating of the sample by the measuring beam.

The spectral output of the xenon lamp was measured by means of the monochromator and a calibrated photodiode (750–850 nm) or PbSe-cell (950–1100 nm). Excitation spectra were calculated by dividing the amplitude of the absorbance changes by the number of incident photons at each wavelength.

Fluorescence excitation spectra were measured as described in Ref. 12.

Results

Heliobacterium chlorum

Membrane fragments of *H. chlorum* were excited with short flashes at selected wavelengths. Figs. 1A and B show the kinetics of absorbance changes at 465 nm induced by a saturating laser flash at 532 nm. At this wavelength the initial absorbance increase is caused by the formation of a charge separated state in the reaction center [6,8]. At room temperature the absorbance increase is followed by a decay with a time constant of 6 ms; at 6 K a decay of the initial absorbance increase

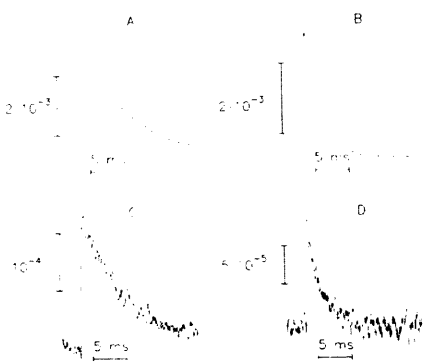

Fig. 1. Kinetics of absorbance changes for *H. chlorum* at 465 nm at 300 K (A and C) and 6 K (B and D); excitation was provided by a saturating laser flash at 532 nm (A and B) or a non-saturating xenon flash at 790 nm (C and D); $A_{-\infty} = 1.1$ at room temperature.

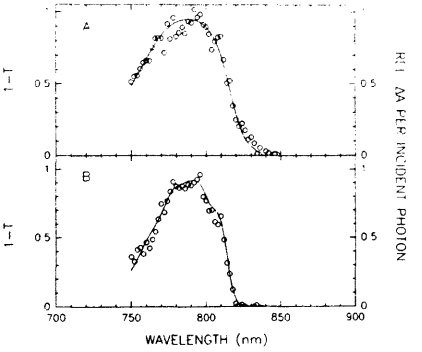

Fig. 2. (A) Absorption $(1-T)$ spectrum of *H. chlorum* membranes (solid line) and excitation spectrum of charge separation, measured at 465 nm (open circles) at 300 K. (B) Same as (A), measured at 6 K. For both spectra the spectral resolution was 5 nm. Further conditions as in Fig. 1.

with a time constant of 2.3 ms is observed. At 6 K the decay is caused by a back reaction of P-798$^+$ with a reduced secondary electron acceptor [8,9], whereas at room temperature it reflects mainly a rereduction of P-798$^+$ by reduced PMS [6]. The same kinetics, but with a strongly reduced amplitude were obtained with weak, monochromatic xenon flashes at 790 nm (Figs. 1C and D). The fraction of reaction centers in which charge separation was induced by the xenon flashes could be calculated by comparing the amplitude of the absorbance change with the maximum value induced by a saturating flash. At room temperature, $4.9 \pm 0.3\%$ of the reaction centers showed charge separation, at 6 K this value was $3.6 \pm 0.4\%$. After correction for the slightly different absorbances at 790 nm at the two temperatures, this yielded a decrease in quantum efficiency by $29 \pm 4\%$ upon cooling from 300 K to 6 K at this wavelength.

The amplitude of the absorbance change induced by a xenon flash was measured likewise for different excitation wavelengths in the Q_y-absorption region. Excitation spectra for charge separation at 300 K and 6 K are shown in Fig. 2 (circles). Fig. 2 also shows the absorption $(1-T)$ spectra of the samples, measured with the same optical arrangement. Within the error of measurement, the excitation spectra are identical to the absorption spectra after normalization at their maxima, both at 300 K and 6 K. It is of special interest to note that there is no decrease in the photochemical efficiency for the red shoulder of the Q_y region at 6 K, which shoulder was attributed to BChl *g*-808 [3,8]. This indicates that the efficiency for excitation transfer from the antenna BChls to the reaction center is essentially the same for all spectral species, BChl *g*-778, BChl *g*-794 and BChl *g*-808, even at 6 K, in agreement with earlier measurements with considerably lower spectral resolution [8].

Rhodopseudomonas viridis

Absorbance difference spectra of membranes of *Rps. viridis*, induced by saturating laser flashes are shown in Fig. 3. The spectrum obtained at room temperature (open circles) resembles that obtained by Holt and Clayton [4] upon illumination with continuous light, except that the main bleaching, due to the oxidation of the primary electron donor (usually called P-960), is located at 980 nm, rather than at 985 nm. At 6 K, this bleaching was shifted to 1010 nm (solid circles). Both spectra showed several bands between 750 and 900 nm, associated with the charge separation in the reaction center. They have been interpreted as electrochromic shifts of reaction center pigments, the appearance of a monomeric absorption band of one of the special pair BChls, and a bleaching of an exciton band of the primary donor [13–15]. The difference spectra are simi-

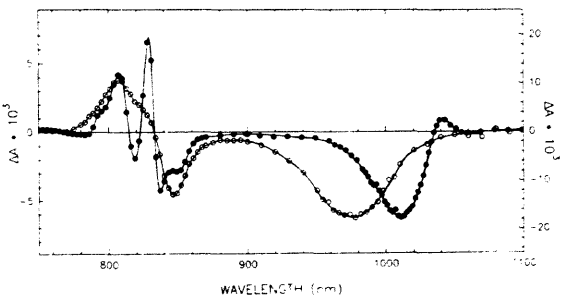

Fig. 3. Spectrum of absorbance changes in membranes of *Rps. viridis* upon a saturating laser flash at 300 K (open circles, left hand scale) and 6 K (solid circles, right hand scale). $A_{1010} = 0.45$ at room temperature.

far to those obtained with isolated reaction centers in this region, but the main bleaching band of the primary electron donor in reaction centers is located at shorter wavelengths (960 nm at 300 K and 990 nm at 10 K [14–16]). We conclude that, although P-960 absorbs at longer wavelengths in membranes than in isolated reaction centers, the wavelength of maximum absorption (980 nm and 1010 nm at 300 K and 6 K, respectively) is still significantly below that of the maximum antenna absorption in the near-infrared, which is at 1015 nm and 1035 nm, respectively (Ref. 4; see also Fig. 5).

The low temperature difference spectrum showed a small positive band at 1040 nm. We ascribe this band to a small change in antenna absorption caused by an electrochromic effect of the charge separation. A similar feature is also observed in the low temperature difference spectrum of *H. chlorum* membranes [8,9].

The relative efficiency of the charge separation at 6 K was measured in a similar way as for *H. chlorum* membranes (see above). Fig. 4 shows the kinetics of the absorbance changes at 807 nm at room temperature and at 6 K induced by either a saturating laser flash or a weak xenon flash. The wavelength of the latter flash was 1020 nm, where the absorption of the sample is approximately the same at both temperatures. The kinetics of the absorbance changes were the same for both flashes. At 300 K the initial absorbance increase was followed by a decay of several hundreds of milliseconds; at 6 K the decay had a monoexponential time constant of 6 ms, presumably due to a back reaction of P-960$^+$ with Q_A^-, the reduced secondary acceptor [17]. Comparison of the amplitudes of the absorbance changes with saturating and non-saturating flashes yielded a relative efficiency of $45 \pm 5\%$ at 6 K

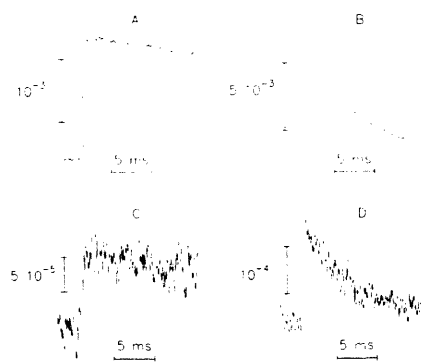

Fig. 4. Kinetics of absorbance changes for *Rps. viridis* at 807 nm at 300 K (A and C) and 6 K (B and D); excitation was provided by a saturating laser flash at 532 nm (A and B) or a non-saturating xenon flash at 1020 nm (C and D); $A_{807} = 0.45$ at room temperature.

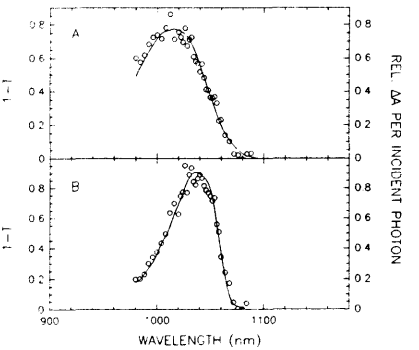

Fig. 5. (A) Absorption ($1-T$) spectrum of *Rps. viridis* membranes (solid line) and excitation spectrum of charge separation, measured at 807 nm, (open circles) at 300 K. (B) Same as (A), measured at 6 K. For both spectra the spectral resolution was 5 nm.

for excitation at 1020 nm, as compared to the efficiency at 300 K.

Excitation spectra for the photochemical efficiency in the Q_y region of BChl *b* are shown in Fig. 5. The spectra were obtained in the same way as described for *H. chlorum*. After normalization in the maximum the excitation spectra were essentially the same as the $1-T$ spectra, both at 300 K and at 6 K. This means that at both temperatures the efficiency of charge separation is constant for the entire Q_y absorption region, as in *H. chlorum*.

A drop by about 55% in the quantum efficiency upon cooling to 6 K was also observed upon excitation with 1064 nm laser flashes, although the uncertainty in the measured values of the absorbance at this wavelength is rather large.

In Fig. 6 the fluorescence excitation spectrum of *Rps. viridis* membranes is compared to the absorption spectrum in the region between 550 nm and 900 nm at 6 K. It is evident that essentially no fluorescence is emitted by the antenna upon excitation of the reaction center pigments absorbing at 780–860 nm, whereas the Q_y band of antenna BChl *b* around 600 nm is clearly seen in the excitation spectrum. We conclude that there is virtually no chance for an excitation to return to the antenna upon excitation of the reaction center.

Discussion

The yield of photochemistry

The results presented in this paper show that energy transfer from antenna BChl to the reaction center in *H. chlorum* and *Rps. viridis* occurs with surprising efficiency even at 6 K. In *H. chlorum* the efficiency decreased by only about 30% upon cooling from 300 K

to 6 K; in *Rps. viridis* the decrease amounted to 55%. A similar decrease had earlier been observed in *Rhodospirillum rubrum* by Rijgersberg et al. [18], who attributed the decrease to a lowered efficiency of energy transfer between antenna BChls. However, the observation that in *H. chlorum* even at 6 K energy transfer from the short-wavelength absorbing BChls to BChl *g*-808 is quite rapid [11,19], indicates that, at least in this species, the effect must be due to a lowering of the efficiency of energy transfer from the antenna to the reaction center, and we shall assume that this is also the case in *Rps. viridis*. For both species the action spectrum for charge separation was found to match the absorption spectrum closely, at 300 K as well as at 6 K. For *H. chlorum* this strongly indicates that all excitations that eventually reach the reaction center have to pass via the long-wavelength absorbing BChl *g*-808, independent of the energy of the absorbed photons.

At 6 K the fluorescence spectrum of *H. chlorum* shows a maximum near 817 nm [3,20], whereas the primary electron donor absorbs maximally near 794 nm [8,9]. For *Rps. viridis* these numbers are 1060 nm [5] and 1010 nm, respectively. In view of the correspondingly small values for the Förster overlap integrals, the efficiency of charge separation would appear to be surprisingly high in both species at low temperature. However, it should be noted that a low rate of energy transfer does not neccesarily imply a very low efficiency. The quantum efficiency of photochemical trapping upon excitation of the antenna, ϕ, can be written as [2]:

$$\phi = k_0/(k_0 + k_1), \qquad (1)$$

where k_0 is the average rate constant of the de-excitation in the antenna due to photochemical trapping in the reaction center and k_1 the rate constant of excitation decay in the absence of reaction centers, determined by various loss processes, including fluorescence. For isolated antenna-reaction center complexes of heliobacteria a value of 25 ps has been reported for the lifetime of excitations in the antenna at room temperature [21]. A similar value appears to apply to membranes (Van Noort, P, personal communication). Assuming $k_0 = (25\ \text{ps})^{-1}$ and $k_1 = 1\ \text{ns}^{-1}$, the same value as obtained for isolated antenna complexes of *Rb. sphaeroides* [22], Eqn. 1 yields a value of 97.6% for the absolute quantum yield of excitation transfer to the reaction center at room temperature. Using this value for the quantum yield at 300 K our results give a yield of 69% at 6 K. Again assuming $k_1 = 1\ \text{ns}^{-1}$, this gives a value of $(440\ \text{ps})^{-1}$ for k_0 at this temperature. By the same reasoning, the fluorescence yield with open reaction centers should increase by a factor of about 13 upon cooling, in reasonable agreement with experimental observations [3]. At 10 K a component of about 200 ps was observed in the decay of excited BChl g-808 [11,19] under conditions where all or nearly all of the reaction centers contained P-798 in the oxidized state. In view of the observation that P-798 is approximately twice as efficient a quencher for antenna fluorescence in the oxidized than in the reduced state [20], these observations appear to fit well with our calculation. On the other hand, the rapid photooxidation of P-798 ($\lesssim 20$ ps) at 15 K observed upon flash excitation at 532 nm [11,19] would not appear to agree with a low rate of energy transfer to the reaction center. However, these experiments were performed with relatively strong flashes. For a valid comparison subsaturating flashes should be used, especially since quanta at 532 nm are directly absorbed by BChl *g* [23] and therefore at high flash intensity direct excitation of the reaction center may play a significant role in P-798 oxidation.

For *Rps. viridis* k_0 was reported to be $(45\ \text{ps})^{-1}$ [24]

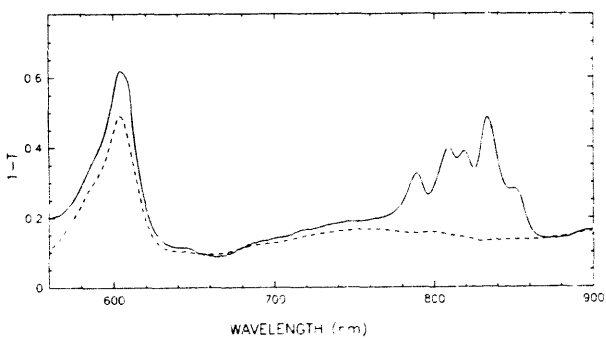

Fig. 6. Solid line: absorption $(1 - T)$ spectrum of *Rps. viridis* membranes at 6 K. Broken line: fluorescence excitation spectrum of the same sample, normalized at 900 nm. The detection wavelength was 1060 nm

or (60 ps)$^{-1}$ (Van Grondelle, R., personal communication). Applying these numbers to Eqn. 1 and again assuming $k_t = 1$ ns^{-1}, independent of temperature, this gives a quantum efficiency for charge separation of 94–96%, in good agreement with the value of 97 ± 7% reported by Trissl et al. [24]. A decrease of this yield to 43% at 6 K then gives $k_u = (1.3$ ns$)^{-1}$. Olson and Clayton [25] had earlier concluded, from action spectra of cytochrome and P-960 oxidation, that the quantum yield of charge separation at room temperature would be less than 70% upon excitation in the antenna. This number seems unlikely, since it would imply a fluorescence lifetime of > 400 ps. No experimental data on the lifetime of excited antenna BChl *b* at liquid helium temperature have been reported so far. It may be noted that an increase of the fluorescence yield by a factor of only 2 was observed upon cooling to 6 K [26], whereas one would expect the decrease in photochemical efficiency to be reflected by a much stronger fluorescence increase.

Rates of energy transfer between antenna and reaction center

Even if we assume that, at low temperature, the rate of energy transfer from neighboring antenna BChls to the reaction center, k_{RC}, is much smaller than that for energy transfer between antenna BChls, k_h, the average rate constant k_u is still in principle a function not only of k_{RC}, but also of the rate constant for photochemical trapping in the reaction center and of the rate constant for energy transfer from the reaction center to neighboring antenna BChls, k_{-RC}. However, reaction center bands in the region 780–860 nm are virtually absent in the excitation spectrum for BChl *b* fluorescence (Fig. 6). A relatively low efficiency of fluorescence at room temperature upon excitation of the reaction center at 830 nm was already observed by Olson et al. [25,27], and similar observations have been reported for other purple bacteria [28,29] and for *Chloroflexus aurantiacus* [30]. There is evidence that energy transfer from accessory BChls to the primary electron donor is quite efficient, even at low temperature [18,31]. Thus, our measurements indicate that the efficiency of energy transfer from the reaction center to the antenna is, at least in *Rps. viridis* at 6 K, at most a few percent. It is clear that this provides optimal conditions for efficient photochemistry, even if k_{RC} is low. For *H. chlorum* too, a low fluorescence efficiency has been observed at 6 K upon excitation in the reaction center band at 670 nm [7].

With $k_{RC} \ll k_h$ and in the absence of back transfer from the reaction center, $k_{RC} \approx k_u$. We tried to estimate whether the value of (440 ps)$^{-1}$ for k_{RC} in *H. chlorum* at 6 K would be compatible with the Förster mechanism. In the absence of evidence to the contrary, we assume that the absorption spectrum of P-798 and the fluorescence spectrum of BChl *g*-808 are essentially homogeneously broadened. Since the absorption difference spectrum of P-798 oxidation was not suitable because of an unknown contribution by the band shift on the long-wavelength side of the bleaching band at low temperature, we used the triplet-minus-singlet difference spectrum [9] to obtain a minimum estimate for the overlap integral with the fluorescence spectrum [3,20] at 6 K. Applying the Förster equation, assuming the orientation factor $\kappa = 1$ and the refractive index $n = 1.5$ [32], this yielded a value for R, the donor-acceptor distance, of 1.8 nm. This value is presumably a weighted average, depending on the arrangement and orientation of the BChl *g*-808 molecules in the vicinity of the reaction center. It is significantly smaller than the estimated value of about 3 nm found for the purple bacterium *Rhodobacter sphaeroides* [32], but this may be related to the fact that in heliobacteria the antenna and reaction center pigments are thought to be bound to the same protein [7,21]. At 200 K the overlap between antenna emission and primary donor absorption was larger by a factor of about 150, but the contribution by BChl *g*-808 to the emission spectrum is not known. We conclude that a significant efficiency for charge separation at 6 K may be explained by the Förster mechanism, if it is assumed that the bands of the pigments involved are homogeneously broadened. For *Rps. viridis* the overlap integral at 6 K may be of the same order of magnitude as for *H. chlorum*, as judged from the spectra of Den Blanken et al. [5], but even a rough estimate of the overlap integral was not possible in this case.

It may be noted that for an energy difference of about 200 cm^{-1}, as calculated from the corresponding absorption maxima, the Boltzmann equilibrium would predict an extremely low ratio for the rate constants of forward and backward energy transfer between antenna and reaction center. However, the assumption of homogeneous broadening of antenna and reaction center bands implies a Förster resonance in the region where they have similar energies. Since detailed knowledge of the electronic band structures is lacking, a simple application of the Boltzmann equilibrium equation is therefore not possible.

Finally, the question arises if the rate of charge separation, which is (0.7 ps)$^{-1}$ in isolated reaction centers of *Rps. viridis* at 8 K [33] is high enough to explain the virtual absence of energy transfer ($\leq 2\%$) from the excited primary electron donor to the antenna. The rate constant for back transfer from the reaction center to the antenna, k_{-RC}, is obviously much larger than for the reverse process, because of the much larger Förster overlap integral. If we assume that the weighted distance between the neighboring antenna BChl(s) and P-960 is 3 nm, as was estimated

for *Rb. sphaeroides* [32], $k_{RC}^{-1} = (1.3\ ns)^{-1}$ would yield a critical distance $R_0 = 4.6$ nm for energy transfer to the reaction center. With $R_0 = 9$ nm for the reverse process, corresponding to a 60-fold larger overlap integral for back transfer, we obtain a modest value of $(25\ ps)^{-1}$ for k_{-RC}^{-1}. This means that it may not be necessary to assume that the primary charge separation is preceded by an even faster relaxation process [29,34] to prevent 'wasteful' loss of energy by back transfer to the antenna.

Acknowledgements

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References

1 Van Grondelle, R. and Amesz, J. (1986) in Light Emission by Plants and Bacteria (Govindjee, Amesz, J. and Fork, D.C., eds.), pp. 191–208, Academic Press, Orlando.
2 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147–195.
3 Van Dorssen, R.J., Vasmel, H. and Amesz, J. (1985) Biochim Biophys. Acta 809, 199–203.
4 Holt, A.S. and Clayton, R.K. (1965) Photochem. Photobiol. 4, 829–831.
5 Den Blanken, H.J., Jongenelis, A.P.J.M. and Hoff, A.J. (1983) Biochim. Biophys. Acta 725, 472–482.
6 Smit, H.W.J., Amesz, J. and Van der Hoeven, M.F.R. (1987) Biochim. Biophys. Acta 893, 232–240.
7 Van de Meent, E.J., Kleinherenbrink, F.A.M. and Amesz, J. (1990) Biochim. Biophys. Acta 1015, 223–230.
8 Smit, H.W.J., Van Dorssen, R.J. and Amesz, J. (1989) Biochim. Biophys. Acta 973, 212–219.
9 Kleinherenbrink, F.A.M., Aartsma, T.J. and Amesz, J. (1991) Biochim. Biophys. Acta 1057, 364–352.
10 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell. Comp. Physiol. 49, 25–68.
11 Van Kan, P.J.M. (1991) Doctoral Thesis, University of Leiden.
12 Otte, S.C.M., Van der Heiden, J.C., Plemme, N. and Amesz, J. (1991) Photosynth. Res. 28, 77–87.
13 Vermeglio, A. and Paillotin, G. (1982) Biochim. Biophys. Acta 681, 32–40.
14 Shuvalov, V.A. and Asadov, A.A. (1979) Biochim. Biophys. Acta 545, 296–308.
15 Thornber, J.P., Cogdell, R.J., Seftor, R.E.B. and Webster, G.D. (1980) Biochim. Biophys. Acta 593, 60–75.
16 Parot, P., Thiery, A. and Vermeglio, A. (1988) in The Photosynthetic Bacterial Reaction Center (Breton, J. and Vermeglio, A., eds.), pp. 251–258, Plenum Press, New York.
17 Shopes, R.J. and Wraight, C.A. (1987) Biochim. Biophys. Acta 893, 409–425.
18 Rijgersberg C.P., Van Grondelle R. and Amesz, J. (1983) Biochim. Biophys. Acta 592, 53–64.
19 Van Kan, P.J.M., Aartsma, T.J. and Amesz, J. (1989) Photosynth. Res. 22, 61–68.
20 Deinum, G., Kramer, H., Aartsma, T.J., Kleinherenbrink, F.A.M. and Amesz, J. (1990) Biochim. Biophys. Acta 1058, 339–344.
21 Trost, J.T. and Blankenship, R.E. (1989) Biochemistry 28, 9898–9904.
22 Sebban, P., Jolchine, G. and Mayaux, J. (1984) Photochem. Photobiol. 39, 247–253.
23 Kobayashi, M., Van de Meent, E.J., Erkelens, C., Amesz, J., Ikegami, I. and Watanabe, T. (1991) Biochim. Biophys. Acta 1057, 89–96.
24 Trissl, H.-W., Breton, J., Deprez, J. and Leibl, W. (1990) Biochim. Biophys. Acta 1015, 322–333.
25 Olson J.M. and Clayton, R.K. (1969) Photochem. Photobiol. 5, 655–660.
26 Deinum, G. (1991) Doctoral Thesis, University of Leiden.
27 Olson, J.M. and Nadler, K.D. (1965) Photochem. Photobiol. 4, 783–788.
28 Wang, R.T. and Clayton, R.K. (1971) Photochem. Photobiol. 13, 215–224.
29 Abdourakhmanov, I.A., Danielius, R.V. and Razjivin, A.P. (1989) FEBS Lett. 245, 47–50.
30 Vasmel, H., Van Dorssen, R.J., De Vos, G.J. and Amesz, J. (1986) Photosynth. Res. 7, 281–294.
31 Vasmel, H., Meiburg, P.F., Kramer, H.J.M., De Vos, J.J. and Amesz, J. (1983) Biochim. Biophys. Acta 724, 333–339.
32 Bergstrom, H., Van Grondelle, R. and Sundstrom, V. (1989) FEBS Lett. 250, 503–508.
33 Fleming, G.R., Martin, J.L. and Breton, J. (1988) Nature 333, 190–192.
34 Meech, S.R., Hoff, A.J. and Wiersma D.A. (1985) Chem. Phys. Lett. 121, 287–292.