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Energy transfer between the reaction center and the antenna in purple bacteria

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The efficiency of energy transfer from the reaction center to the antenna in chromatophores of various species of purple bacteria was estimated from the contribution of the reaction center bands to the excitation spectra of antenna fluorescence. In all species studied, except for wild-type *Rhodobacter sphaeroides*, where the results were inconclusive, the efficiency at 6 K was lower than 5%, and in most cases even lower than 2%. Similar results were obtained with *Rhodopseudomonas viridis* at room temperature. These results indicate that the so-called "trap-limited" model for energy transfer does not apply to purple bacteria: it is concluded that the rate of photochemistry is determined by the rate of energy transfer from the antenna to the reaction center and that back transfer to the antenna cannot compete with the early events of charge separation. Upon blocking of the charge separation in *Rps. viridis* by prior reduction of the primary electron acceptor extensive energy transfer from the reaction center to the antenna occurred.

Introduction

Models of energy transfer in photosynthetic antennas are often based on the assumption that the energy moves freely in and out of the reaction center [1–7]. Such models are called trap-limited, since they imply that the average rate of energy transfer from the antenna to the reaction center is fast as compared to the rate of trapping, defined here as the rate of charge separation. Thus, in the ideal situation the antenna and reaction center after photon absorption will rapidly convert into a state of thermal equilibrium which dissipates its energy by charge separation and subsequent electron transfer processes. The opposite case is the diffusion-limited or migration-limited model, where the rate of photochemistry is largely determined by the time required for an excitation to reach the reaction center [8,9]. The first model applies when the distance between the reaction center pigments and the neighboring antenna pigments is approximately the same as that between two antenna pigments, and when the 'hopping rate', i.e., the rate of energy transfer between two neighboring pigments is much higher than the rate of charge separation. This may be the case for the core of Photosystem I [10] and of green sulfur bacteria and for heliobacteria.

However, for Photosystem II, purple bacteria and green filamentous bacteria the reaction center pigments are located on a separate pigment protein complex. This means that the distance between the reaction center pigments and the nearest neighbors in the antenna may be relatively large and consequently the rates of energy transfer from neighboring antenna pigments to the reaction center low as compared to the hopping rates in the antenna. When the rate of charge separation is fast as compared to the rate of energy transfer to the reaction center, this would result in a special case of the diffusion-limited model, where the rate of trapping *by* (not *in*) the reaction center is the rate-limiting step. In purple bacteria the rate constants of hopping are approximately 10^{12} s^{-1} [11,12] and those of charge separation are (3 to 10) $\cdot 10^{11}$ s^{-1} [13,14], whereas there is evidence that the average rate of energy transfer to the reaction center is approximately $2 \cdot 10^{10}$ s^{-1} [11,15], i.e., more than an order of magnitude lower. If the rate of energy transfer from

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P, primary electron donor; Q_A and Q_B, first and second acceptor quinones; RC, reaction center.

the reaction center to the antenna is of comparable magnitude, this means that this process cannot compete very efficiently with charge separation.

More than 20 years ago, Clayton and co-workers [16,17] and Zankel [18] showed that excitation of the accessory BChls in the reaction center of *Rhodospirillum rubrum*, and *Rhodobacter sphaeroides* R-26 produced relatively little BChl fluorescence, which indicated that excitation energy caught by the reaction center has only a small chance of escaping to the antenna. Similar observations were made by Vasmel et al. [19] with membranes of the green filamentous bacterium *Chloroflexus aurantiacus* at 4 K, while kinetic measurements with chromatophores of *R. rubrum* [20] and with an antenna-reaction center complex from *Chromatium minutissimum* [21] likewise indicated that the excitation energy has only a low probability to be transferred from the reaction center to the antenna.

With a few exceptions [22,23] these observations appear to have been largely overlooked in the literature, and not to have been incorporated in the models for energy migration mentioned above. For this reason we have made a quantitative study of the fluorescence excitation spectra of a number of different species of purple bacteria. The results indicate a very low probability for energy transfer from the photoactive reaction center to the antenna. At 6 K, this probability did not exceed 5% and in most cases could be determined to be less than 2%. Possible causes for this effect are discussed.

Materials and Methods

Rhodopseudomonas viridis strain F, *Rhodospirillum rubrum* strain S1, *Rhodobacter sphaeroides* strain 2.4.1. (wild-type) and *Rb. sphaeroides* mutant R-26 were all grown in a medium described in Ref. 24. The cells were harvested by centrifugation and resuspended in 10 mM Tris-HCl buffer (pH 8.0). Cells of *Chromatium tepidum*, grown as described in Ref. 25, were a kind gift of Dr. A. Verméglio, CEN Cadarache. The cells were incubated with 2 mg DNAase (Desoxyribonuclease I) per 100 ml cell suspension during 15 min at room temperature to reduce the viscosity of the suspension during and after sonication. Then the cells were sonicated for 20 min at 0 °C, and subsequently centrifuged for 10 min at $12\,000 \times g$ to remove unbroken cells and large cell fragments. Membranes were collected by ultracentrifugation at $223\,000 \times g$ for 90 min. The pellet was resuspended in 10 mM Tris-HCl buffer (pH 8.0). If necessary, 10 mM ascorbate was added to keep the primary electron donor in the reduced state.

Absorption, fluorescence emission and fluorescence excitation spectroscopy were performed using a single beam spectrophotometer [26,27], equipped with a personal computer for data analysis. The spectral resolution was 1.5 nm for absorption measurements and 2.5 nm for fluorescence measurements. The intensity of the excitation light was less than 50 $\mu W/cm^2$. To obtain clear samples at low temperatures, glycerol (60% v/v) was added to the samples.

The position of the main absorption bands and the fluorescence emission maxima of the chromatophores of the purple bacteria studied are given in Table I.

Results

Rhodopseudomonas viridis

Fig. 1 shows the absorption $(1 - T)$ spectrum of *Rps. viridis* membranes at room temperature (solid line). Absorption bands at 780–850 nm arise from reaction center pigments, and the band at 604 nm is due to the Q_x transition of BChl *b*. The fluorescence excitation spectrum (dashed line) showed no measurable contribution by the reaction center pigments, indicating that essentially no energy transfer from the reaction center to the antenna occurred. Both spectra did not change upon the addition of 10 mM *o*-phenanthroline, which has been shown to block electron transfer from Q_A to Q_B, resulting in a considerably shorter lifetime of P^+ [28]. This indicates that the primary electron donor P was in the reduced state during the measurements.

TABLE I

The locations of the maxima of the major BChl Q_x and Q_y absorption and fluorescence emission bands at 6 K of membrane fragments of the purple bacteria studied.

For *Rps. viridis*, the room temperature data are also given (italics). The relative amplitudes are indicated in parentheses.

Species	Absorption (nm)					Fluorescence (nm)
Rps. viridis	605 (0.09)		834 (0.06)		1038 (1.00)	1058
	604 (0.26)		*832 (0.19)*		*1015 (1.00)*	*1040*
R. rubrum	591 (0.11)		804 (0.10)		896 (1.00)	917
Rb. sphaeroides R-26	592 (0.15)		804 (0.22)		883 (1.00)	907
Rb. sphaeroides WT	588 (0.44)		799 (1.81)	856 (2.17)	889 (1.00)	908
C. tepidum	594 (0.51)	796 (0.93)	808 (1.02)	871 (1.57)	938 (1.00)	967

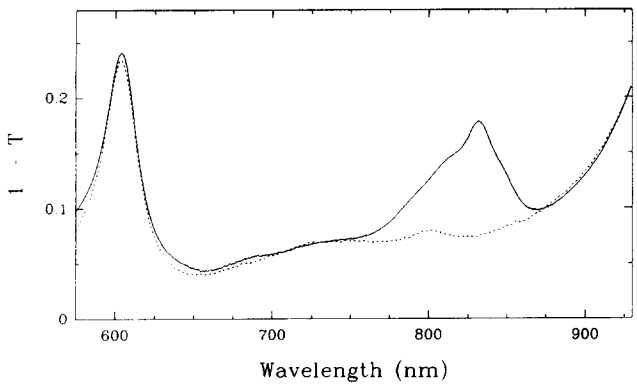

Fig. 1. Solid line: absorption $(1-T)$ spectrum of *Rps. viridis* membranes at room temperature. Dashed line: fluorescence excitation spectrum of the same sample, normalized at the Q_y antenna band (1015 nm). The detection wavelength was 1040 nm.

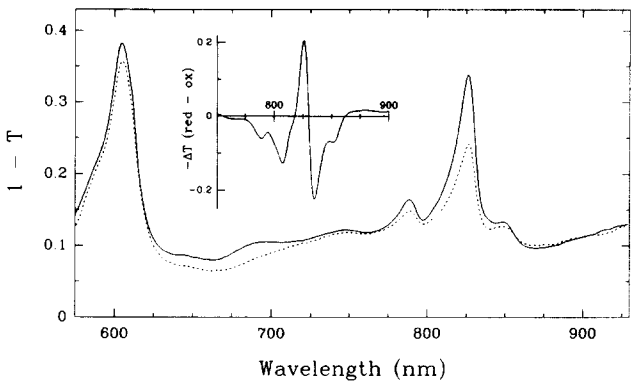

Fig. 3. Solid line: absorption $(1-T)$ spectrum of *Rps. viridis* membranes at 6 K upon addition of 10 mM sodium dithionite and actinic illumination during cooling. Dashed line: fluorescence excitation spectrum of the same sample, normalized at 1038 nm. The detection wavelength was 1060 nm. Inset: the absorption difference spectrum for reduced minus oxidized BPh at 6 K (Fig. 3 minus Fig. 2, solid lines) after normalization to an absorption of 0.62 at the Q_x band.

The low probability of energy transfer from the reaction center to the antenna of *Rps. viridis* is even better illustrated by spectra measured at 6 K (Fig. 2). Whereas the room temperature absorption spectrum showed little structure near 820 nm, five reaction center bands could be discerned at low temperature, located at 789, 808, 819, 833 and 853 nm according to the second derivative spectrum (Fig. 2A and B, solid lines). The same bands, but with different relative amplitudes have been observed in the absorption spectrum of isolated reaction centers [29]. They may be attributed to the two BPhs and the two accessory BChls of the reaction center and to the high energy exciton band of the primary electron donor P [29]. No contribution of any of these bands was observed in either the fluorescence excitation spectrum for antenna emission (Fig. 2A, dashed line) or in its second derivative (Fig. 2B), indicating a very efficient trapping of excitation energy

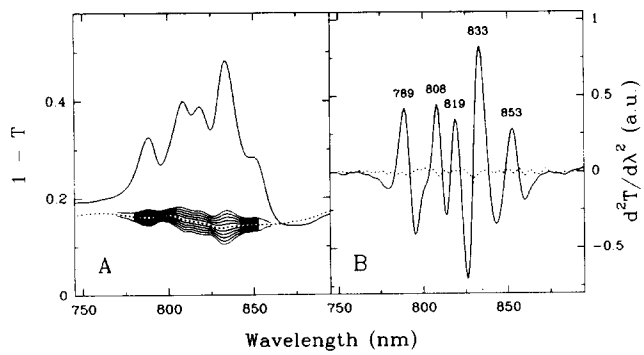

Fig. 2. (A) Solid line: absorption $(1-T)$ spectrum of *Rps. viridis* membranes at 6 K. Dashed line: fluorescence excitation spectrum of the same sample, normalized at 1038 nm (adapted from Ref. 41). The detection wavelength was 1060 nm. For comparison, the thin lines give spectra obtained by the addition or subtraction of a component corresponding to 2, 4, 6, 8 and 10% efficiency for energy transfer from the RC to the antenna, respectively. (B) Second derivatives of the absorption and fluorescence excitation spectra (solid and dashed lines, respectively).

in the reaction center. From a comparison of the measured excitation spectrum with spectra to which a known contribution by energy transfer was added (thin lines) it may be concluded that the efficiency of energy transfer from the reaction center to the antenna was at most 2%. The same conclusion follows from the second derivatives.

We also performed experiments under conditions where the primary electron acceptor, BPh *b*, was in the reduced state. The first acceptor quinone, Q_A, was reduced by the addition of 10 mM sodium dithionite. Upon actinic illumination of the sample during cooling the primary electron acceptor was accumulated in the reduced state, thus inhibiting charge separation [30]. Absorption $(1-T)$ and fluorescence excitation spectra at 6 K for this case are given in Fig. 3. In the region of 775–850 nm, the absorption spectrum (solid line) was drastically changed as compared to that of Fig. 2, with bands at 789, 826 and 849 nm and a weak shoulder near 810 nm, indicating essentially complete reduction of the primary acceptor under these conditions. The absorption spectrum clearly resembled that of isolated reaction centers with photoreduced BPh [29], as is also confirmed by the strong similarity of the corresponding difference spectrum of our membranes (Fig. 3, inset) to that of isolated reaction centers [29]. The fluorescence excitation spectrum now showed a clear contribution by the reaction center bands, indicating extensive energy transfer from the reduced reaction center to the antenna.

BChl a containing purple bacteria

Irreversible trapping of excitation energy by the reaction center was not confined to *Rps. viridis*. Fig. 4 shows the absorption and excitation spectra of mem-

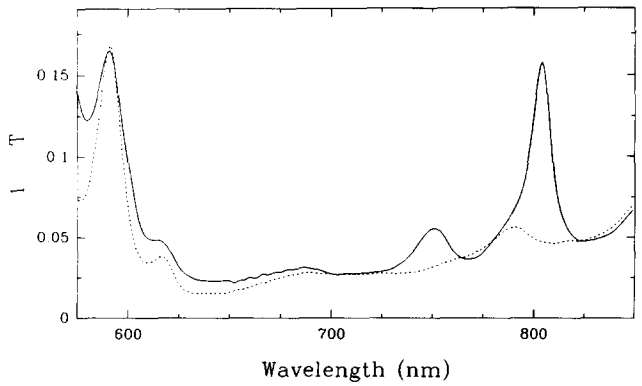

Fig. 4. Solid line: absorption $(1-T)$ spectrum of *R. rubrum* membranes at 6 K. Dashed line: fluorescence excitation spectrum of the same sample, normalized at the Q_y antenna band (896 nm). The detection wavelength was 917 nm.

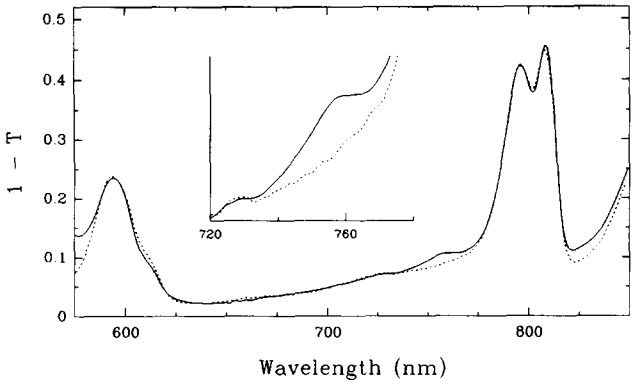

Fig. 6. Solid line: absorption $(1-T)$ spectrum of *C. tepidum* membranes at 6 K. Dashed line: fluorescence excitation spectrum of the same sample, normalized at the Q_y band at 871 nm. The detection wavelength was 910 nm. The inset shows the region of the reaction center BPhs on a 5-fold expanded scale.

branes of *R. rubrum* at 6 K. The absorption spectrum is characterized by the Q_x band of BChl *a* at 590 nm, a band near 750 nm from the reaction center BPhs, a strong contribution from the reaction center BChls at 804 nm, and a shoulder near 790 nm. Both reaction center bands show no trace of contribution to the antenna emission, indicating the virtual absence of back transfer of excitation energy from the reaction center to the antenna. The shoulder at 790 nm is probably a vibrational band of the antenna BChls, as suggested by its presence as a well-resolved band in the excitation spectrum.

Similar results were obtained with the carotenoidless mutant R-26 of *Rb. sphaeroides* (Fig. 5). The mutant is characterized by the absence of carotenoids and peripheral antenna complexes, the only remaining antenna BChl *a* absorbing at 883 nm at 6 K. Whereas the Q_x band near 590 nm is clearly visible in the fluorescence excitation spectrum, no contribution by the reaction center BPhs and BChls (absorbing near 755 and 805 nm, respectively) could be detected. As in *R. rubrum* the band at 780 nm, which contributes efficiently to the antenna fluorescence, is probably a vibrational band of the antenna BChl.

Fig. 6 shows the absorption and fluorescence excitation spectra of *C. tepidum*, a thermophilic purple sulfur bacterium [25]. In addition to the core complex, B920, this species contains at least one peripheral antenna complex, B800–850 [31]. At 6 K the 800 nm band is resolved in two components, at 796 and 808 nm (Table I and Ref. 31). Reaction center bands near 800 nm are therefore completely masked by a strong absorption at this wavelength, and the only reaction center band that can be resolved in the absorption spectrum is a relatively weak BPh *a* band at 754 nm. As in *Chloroflexus aurantiacus* [19], an indication for the efficiency of energy transfer from the reaction center to the antenna is therefore only given by the relative amplitude of the band of reaction center BPhs. This band is clearly missing in the excitation spectrum, indicating the vir-

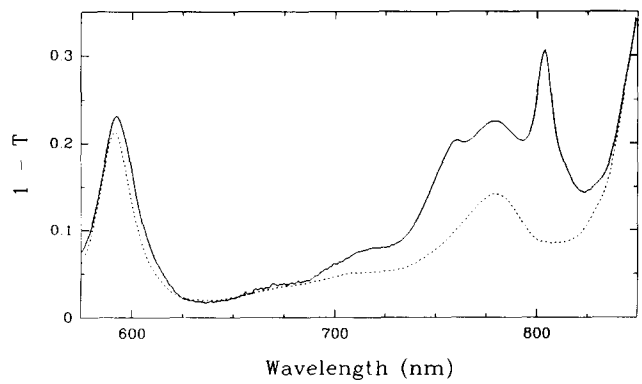

Fig. 5. Solid line: absorption $(1-T)$ spectrum of *Rb. sphaeroides* R-26 membranes at 6 K. Dashed line: fluorescence excitation spectrum of the same sample, normalized at 883 nm. The measurements were done in the presence of 10 mM ascorbate; the detection wavelength was 910 nm.

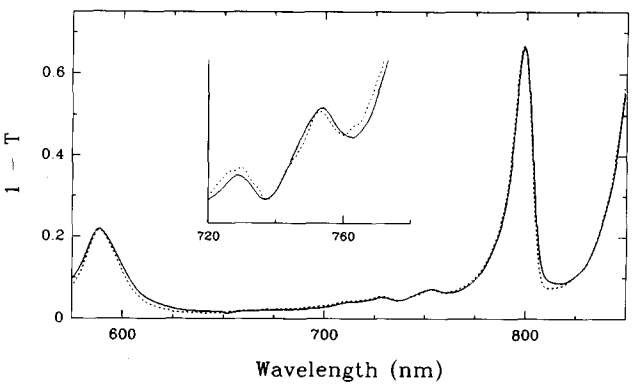

Fig. 7. Solid line: absorption $(1-T)$ spectrum of *Rb. sphaeroides* WT membranes at 6 K. Dashed line: fluorescence excitation spectrum of the same sample, normalized at 856 nm. Ascorbate (10 mM) was present; the detection wavelength was 910 nm. The inset shows the region of the reaction center BPhs on an 8-fold expanded scale.

tual absence of energy transfer from the reaction center to the antenna, as in the other species.

Inconclusive results were obtained with wild-type *Rb. sphaeroides*. At first sight the presence of a 754 nm band in the excitation spectrum (Fig. 7) would indicate an efficient back transfer from the reaction center to the antenna. However, the low temperature absorption spectrum of the isolated B800–850 complex shows a band at precisely this wavelength of approximately the same relative amplitude [32]. Moreover, it can be estimated by comparison with the height of the Q_y band of the core antenna (not shown in Fig. 7), that the BPh reaction center band should constitute only about one-third of the 754 nm band in the absorption spectrum. Therefore a definite conclusion concerning the contribution of the reaction center band to the fluorescence excitation spectrum was not possible in this case.

Discussion and Conclusions

As judged from the absence of significant contributions of the reaction center BPhs or BChls to the excitation spectra of antenna fluorescence, in purple bacteria the efficiency of energy transfer from the reaction center to the antenna is very low. This applies to all species studied, except for *Rb. sphaeroides* wild-type, where the results were inconclusive. The efficiencies of energy transfer from the reaction center to the antenna for the various species are summarized in Table II. The numbers were determined from the amplitudes of the reaction center bands in the fluorescence excitation spectrum, if necessary by means of the second derivatives.

Experiments with isolated reaction centers have shown that the rates and efficiencies of energy transfer from the BPhs and the accessory BChls to the primary electron donor P are quite high [33]. Since there is no reason to assume that they will be significantly different when the reaction centers are contained in the membrane, our results must be explained by a very low efficiency for energy transfer from excited P to the antenna. This means that we are dealing with a special case of the diffusion-limited model: the rate of photochemistry is determined by the rate of transfer from the antenna to the reaction center. The excitation spectrum of Fig. 3 shows that a different situation applies when charge separation is blocked by prior reduction of the primary electron acceptor: the reaction center bands are now clearly visible, indicating extensive energy transfer from P to the antenna under these conditions.

TABLE II

Efficiencies of energy transfer from the reaction center to the antenna at 6 K of the purple bacteria studied

Species	Efficiency (%)	Conditions
Rps. viridis	≤ 10	room temperature
	≤ 2	open RCs
	60 ± 2	reduced primary acceptor
R. rubrum	≤ 2	
Rb. sphaeroides R-26	≤ 2	
C. tepidum	≤ 5	

It may be noted that our results with *R. rubrum* and *Rb. sphaeroides* R-26 are in agreement with those obtained by Clayton et al. [16,17] at room temperature, although the accuracy and spectral resolution of the present experiments is higher. However, our results with *Rps. viridis* are at variance with those of Olson and Clayton [34], who concluded that quanta absorbed by the reaction center at 830 nm were about 70% effective in exciting antenna fluorescence in this species. This discrepancy might be due to the different conditions of the experiment. In our experiments the reaction center cytochrome *c* was in the oxidized state in the dark, as was checked by light-induced absorption difference spectroscopy, whereas under the conditions of Olson and Clayton it was in the reduced state. In the latter case, illumination might cause the photoaccumulation of reduced electron acceptors [30], thus promoting energy transfer from the reaction center to the antenna.

Once an excitation has arrived on the primary electron donor, two competing processes occur, namely charge separation, and back transfer of the excitation to the antenna. It is therefore of interest to analyze our results in terms of time constants of energy transfer and charge separation. For isolated reaction centers of *Rps. viridis* a time constant of 2.8 ps has been reported for the primary charge separation at room temperature, which number decreases to a value of 0.7 ps at temperatures below approx. 25 K [14]. At low temperature we observed an efficiency of no more than 2% for energy transfer from the excited reaction center to the antenna, which means that the time constant for energy transfer from the reaction center to the antenna, τ_A, should be at least 35 ps. By a similar calculation we arrive at a value of 30 ps or higher at room temperature.

These values may be compared with those of forward energy transfer. Unfortunately, data for liquid helium temperature are not available. For *Rps. viridis* the lifetime of excited antenna BChl *b* with open reaction centers has been estimated at 215 ps at 25 K [35] and at 50–75 ps at room temperature [35–37]. Correction for an estimated rate constant of 1 ns^{-1} for excitation decay in the absence of reaction centers [35] would yield slightly lower numbers for the average rate of energy transfer to the reaction center, corresponding to time constants of 275 ps and 53–80 ps, respectively.

The interpretation of these numbers depends on the model used for the antenna organization. We assume

that the antenna of *Rps. viridis* is homogeneous [35,38], N, the number of BChls/RC, being equal to 24 [38,39]. On basis of the electron micrographs [39] we further assume that the coordination number q, i.e., the number of equivalent neighboring antenna BChls surrounding the reaction center [36,40], is 6. This means that the 'true' time constant for energy transfer from one of these BChls to the reaction center is $q/N \times 275 = 70$ ps at 25 K and about 15 ps at room temperature. In comparing these numbers with those for τ_A, one has to take into account that the time constants for energy transfer to a single neighboring antenna BChl are $q\tau_A$, yielding numbers of 200 ps or more for low as well as room temperature. If we take into account that the overlap integrals for back transfer to the antenna are almost certainly considerably larger than those for forward transfer, because of the considerable red shift of the antenna with respect to the reaction center [41], a number of 200 ps appears to be improbably high. The same conclusion appears to apply to the other species, although a quantitative comparison is not possible here. It may be noted that the, perhaps less likely, 'monocoordinate' antenna model [40], with $q = 1$, yields the same conclusion, with time constants of about 10 and about 2.5 ps for the forward process at low and room temperature, respectively, and of 30 ps or more for back transfer to the antenna.

The above data might be tentatively explained by assuming that the charge separation, i.e., electron transfer to accessory BChl or BPh, is preceded by rapid relaxation processes in the reaction center which are at least one order of magnitude faster, and which are able to compete effectively with the energy transfer process. Evidence for a rapid relaxation in isolated reaction centers of *Rb. sphaeroides* has been reported recently [42,43]. The same process may be assumed to occur in chromatophores. It should be noted, however, that these relaxation processes should not be effective in 'closed' reaction centers where electron transfer to BPh does not occur (Fig. 3). Hole-burning experiments suggest that this may indeed be the case [44].

Acknowledgments

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References

1 Robinson, G.W. (1967) Brookhaven Symp. Biol. 19, 16–45.
2 Beauregard, M., Martin, I. and Holzwarth, A.R. (1991) Biochim. Biophys. Acta 1060, 271–283.
3 Fischer, M.R. and Hoff, A.J. (1992) Biophys. J. 63, 911–916.
4 Holzwarth, A.R. (1987) in The Light Reactions (Barber, J., ed.), pp. 95–157, Elsevier, Amsterdam.
5 Freiberg, A., Godik, V.I. and Timpmann, K. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. 1, pp. 45–48, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht.
6 Pearlstein, R.M. (1982) in Photosynthesis, Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. 1, pp. 293–329, Academic Press, New York.
7 Borisov, A.Y. (1990) Photosynth. Res. 23, 283–289.
8 Fetisova, Z.G., Borisov, A.Y. and Fok, M.V. (1985) J. Theor. Biol. 112, 41–75.
9 Owens, T.G., Webb, S.P., Mets, L., Alberte, R.S. and Fleming, G.R. (1987) Proc. Natl. Acad. Sci. USA 84, 1532–1536.
10 Holzwarth, A.R. and Roelofs, T.A. (1992) J. Photochem. Photobiol. B: Biol. 15, 45–62.
11 Sundström, V. and Van Grondelle, R. (1990) J. Opt. Soc. Am. B 7, 1595–1603.
12 Shreve, A.P., Trautman, J.K., Frank, H.A., Owens, T.G. and Albrecht, A.C. (1991) Biochim. Biophys. Acta 1058, 280–288.
13 Martin, J.L., Breton, J., Hoff, A.J., Migus, A. and Antonetti, A. (1986) Proc. Natl. Acad. Sci. USA 83, 957–961.
14 Fleming, G.R., Martin, J.L. and Breton, J. (1988) Nature 333, 190–192.
15 Visscher, K.J., Bergström, H., Sundström, V., Hunter, C.N. and Van Grondelle, R. (1989) Photosynth. Res. 22, 211–217.
16 Clayton, R.K. and Sistrom, W.R. (1966) Photochem. Photobiol. 5, 661–668.
17 Wang, R.T. and Clayton, R.K. (1971) Photochem. Photobiol. 13, 215–224.
18 Zankel, K.L. (1969) Photochem. Photobiol. 10, 259–266.
19 Vasmel, H., Van Dorssen, R.J., De Vos, G.J. and Amesz, J. (1986) Photosynth. Res. 7, 281–294.
20 Danielius, R.V. and Razjivin, A.P. (1988) in Ultrafast Phenomena in Spectroscopy (Rudzikas, Z., Piskarskas, A. and Baltramiejunas, R., eds.), pp. 231–239, World Scientific, Singapore.
21 Abdourakhmanov, I.A., Danielius, R.V. and Razjivin, A.P. (1989) FEBS Lett. 245, 47–50.
22 Amesz, J. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 333–340, Plenum Press, New York.
23 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147–195.
24 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell. Comp. Physiol. 49, 25–68.
25 Madigan, M.T. (1984) Science 225, 313–315.
26 Rijgersberg, C.P., Van Grondelle, R. and Amesz, J. (1980) Biochim. Biophys. Acta 592, 53–64.
27 Otte, S.C.M. (1992) Doctoral Thesis, University of Leiden.
28 Halsey, Y.D. and Parson, W.W. (1974) Biochim. Biophys. Acta 347, 404–416.
29 Verméglio, A. and Paillotin, G. (1982) Biochim. Biophys. Acta 681, 32–40.
30 Tiede, D.M., Prince, R.C., Reed, G.H. and Dutton, P.L. (1976) FEBS Lett. 65, 301–304.
31 Garcia, D., Parot, P., Verméglio, A. and Madigan, M.T. (1986) Biochim. Biophys. Acta 850, 390–395.
32 Van Grondelle, R., Kramer, H.J.M. and Rijgersberg, C.P. (1982) Biochim. Biophys. Acta 682, 208–215.
33 Breton, J., Martin, J.L., Migus, A., Antonetti, A. and Orszag, A. (1986) Proc. Natl. Acad. Sci. USA 83, 5121–5125.
34 Olson, J.M. and Clayton, R.K. (1966) Photochem. Photobiol. 5, 655–660.
35 Kleinherenbrink, F.A.M., Cheng, P., Amesz, J. and Blankenship, R.E. (1993) Photochem. Photobiol. 57, 13–18.
36 Zhang, F.G., Gillbro, T., Van Grondelle, R. and Sundström, V. (1992) Biophys. J. 61, 694–703.
37 Bittersmann, E., Blankenship, R.E. and Woodbury, N. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), pp. 169–172, Kluwer, Dordrecht.

38 Deinum, G. (1991) Doctoral Thesis, University of Leiden.
39 Miller, K.R. (1982) Nature 300, 53–55.
40 Pearlstein, R.M. (1992) J. Luminesc. 51, 139–147.
41 Kleinherenbrink, F.A.M., Deinium, G., Otte, S.C.M., Hoff, A.J. and Amesz, J. (1992) Biochim. Biophys. Acta 1099, 175–181.
42 Vos, M.H., Lambry, J.-L., Robles, S.J., Youvan, D.C., Breton, J. and Martin, J.-L. (1992) Proc. Natl. Acad. Sci. USA 89, 613–617.
43 Meech, S.R., Hoff, A.J. and Wiersma, D.A. (1985) Chem. Phys. Lett. 121, 287–292.
44 Shuvalov, V.A., Klevanik, A.V., Ganago, A.O., Shkuropatov, A.Ya. and Gubanov, V.S. (1988) FEBS Lett. 237, 57–60.