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Picosecond energy transfer and trapping kinetics in living cells of the green bacterium *Chloroflexus aurantiacus* [1]

Marc G. Müller, Kai Griebenow and Alfred R. Holzwarth

Max-Planck-Institut für Strahlenchemie, Mülheim a.d. Ruhr (Germany)

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The excitation energy transfer and trapping processes in intact cells of *Chloroflexus aurantiacus* were studied by picosecond time-resolved fluorescence spectroscopy. The fluorescence decay kinetics is investigated over the near infrared emission range between 730 nm and 920 nm using various excitation wavelengths and excitation intensities. The data were analyzed by global decay analysis and are presented as decay-associated spectra (DAS). The specific dependence of the decay kinetics on the excitation wavelength and on the photochemical redox state of the reaction center (RC) allows the identification of the energy transfer and trapping components. The DAS provide evidence for two chlorosomal energy transfer processes. The first one occurs between the chlorosomal bacteriochlorophyll (BChl)-*c* and the BChl-a_{792} complex (B_{792}) in the chlorosomal baseplate with an equilibration time constant of 15–16 ps, while the second one occurs from the B_{792} pigments to the BChl-a_{806} pigments in the $B_{806-866}$complex with a time constant of 35–40 ps. The overall energy trapping process in whole cells is mainly determined by the kinetics of the primary charge separation process in the RCs. With open RCs (Q_A oxidized) the trapping time constant is 70–90 ps, while the trapping process with closed RCs (Q_A reduced) takes as long as 180–200 ps. The results on whole cells reported here are interpreted in conjunction with those reported earlier for the various isolated complexes, i.e., two different chlorosome preparations (Holzwarth, A.R., Müller, M.G. and Griebenow, K. (1990) J. Photochem. Photobiol. B 5, 457–465), the $B_{806-866}$ complex (Griebenow, K., Müller, M.G. and Holzwarth, A.R. (1991) Biochim. Biophys. Acta 1059, 226–232) and isolated reaction centers (Müller, M.G., Griebenow, K. and Holzwarth, A.R. (1991) Biochim. Biophys. Acta 1098, 1–12). Based on these data, a unified and self-consistent scheme for the primary processes in the whole photosynthetic system of *C. aurantiacus* is presented. The BChl antenna pigment groups are arranged to form a linear energy transfer cascade with four energy transfer steps from shorter-wavelength- to longer-wavelength-absorbing antenna pools. The overall fluorescence decay kinetics of the photosynthetic system of *C. aurantiacus* turns out to be 'trap-limited' by the reaction center rather than 'diffusion-limited' by the energy transfer processes.

Introduction

Photosynthetic eubacteria, except cyanobacteria, generally contain several antenna pigment groups of bacteriochlorophylls (BChl) [2,3] to capture light energy [4–7]. In the photosynthetic system of the thermophilic green filamentous bacterium *Chloroflexus aurantiacus* the BChl-*c* chromophores [8] localized in the extramembranous chlorosomes represent the major antenna pigments. The BChl-a_{806} and BChl-a_{866} chromophores of the $B_{806-866}$ complex are located in the cytoplasmic membrane next to the RC, which converts the absorbed energy into an electrochemical potential by charge separation [9–13]. An additional complex of BChl-a_{792} chromophores occurs in the so-called baseplate of the chlorosome and is supposed to optimize the energy transfer from the chlorosome to the antenna pigments in the cytoplasmic membrane. Thus the major antenna system of *C. aurantiacus* has many similarities to those of green sulfur bacteria, like e.g. *Chlorobium limicola* [2,3,11]. The components localized in the cytoplasmic membrane, however, bear many similarities to those of purple bacteria, like e.g.

Correspondence to: A.R. Holzwarth, Max-Planck-Insitut für Strahlenchemie, Stiftstrasse 34–36, D-45470 Mülheim a.d. Ruhr, Germany.

[1] A preliminary account of part of this work has been presented at the International Photosynthesis Congress in Stockholm, 1991, see Ref. 1.

Abbreviations: BChl, bacteriochlorophyll; B_{792}, bacteriochlorophyll-*a*-792 antenna complex; $B_{806-866}$, bacteriochlorophyll-*a*-806-866 antenna complex; DAS, decay-associated spectrum; GEF, gel-electrophoretic filtration; H, bacteriopheophytin active (H_A) in the reaction center; P, special pair of bacteriochlorophyll *a* in the reaction center; SDGC, sucrose density gradient centrifugation; SPT, single-photon timing; Q, quinone; RC, reaction center.

Rhodobacter sphaeroides [2,3,7,14,15]. These similarities comprise both the major cytoplasmic antenna, the $B_{806-866}$ complex, which represents an equivalent of the purple bacterial $B_{800-850}$ complex [16–19], and the RC structure [9,14,15,20,21]. After excitation of the special pair chromophores (P) in the RC by excitons from the antenna systems a primary electron transfer to a bacteriopheophytin-*a* acceptor molecule (H_A) is induced [22]. A secondary electron transfer to a menaquinone molecule (Q_A) [23] follows in order to stabilize the charge separation across the cytoplasmic membrane [24].

Fluorescence decays of living cells of *C. aurantiacus* have been measured in the past by several groups with different temporal resolution [1,25–28]. Although two energy transfer steps with equilibration times of ≈ 15 ps and ≈ 35 ps have been resolved previously [1,27–29] the assignment of the lifetime components associated with the energy trapping process in the RC remained unclear. To clarify these problems, the present study concentrates additionally on the differences in the trapping processes of excitation energy in cells with open (quinone Q_A oxidized) and closed (quinone Q_A reduced) RCs. Furthermore, our previously published results on isolated complexes from *C. aurantiacus* such as GEF and SDGC chlorosomes [29,30], the $B_{806-866}$ complex [16] and the isolated RC [31,32] will be combined in a self-consistent kinetic model with the data from intact cells. Thus this model provides a complete scheme for the primary photophysical and photochemical processes in the intact photosynthetic organism that is supported also by the data on isolated antenna and RC components.

Materials and Methods

C. aurantiacus cells strain Ok-70-fl (obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig) were grown unaerobically in batch cultures at 50°C under low-light conditions as described previously [33,34]. For each series of measurements 1 L batches were transferred anaerobically into an oxygen-tight bottle. The sample was stirred and purged with nitrogen during the measurements and pumped through the fluorescence cuvette via oxygen-tight Tygon® tubings.

Picosecond fluorescence decays were recorded by the single-photon-timing technique upon excitation with a cavity-dumped dye laser system (dye laser 375B and cavity dumper 344S, Spectra Physics) [35]. A repetition rate of 4 MHz was chosen. The dye laser was synchronously pumped by a mode-locked argon ion laser (Model 2030, Spectra Physics). DCM and Styryl 8 were used as laser dyes to generate optical pulses in the wavelength range between 720 nm and 815 nm with a halfwidth of 10–15 ps. During the measurements the sample was pumped through a flow cuvette with a window of 1.5×1.5 mm^2. Fluorescence photons were detected with a fast near-infrared sensitive microchannel plate photomultiplier (R2809U-05, Hamamatsu) employing a near-infrared double monochromator (DH 10.VIR, Jobin Yvon, bandwidth ≈ 12 nm) to select the emission wavelength. The full width at half maximum of the system temporal response function was 35–40 ps. Generally, fluorescence decay measurements were performed under magic angle polarisation conditions with a high signal to noise ratio corresponding to 20 000–30 000 counts in the peak channel. Data analysis was performed by a global analysis procedure using a sum of exponentials as model function [36]. The wavelength dependence of the amplitudes was plotted as decay-associated spectra (DAS). The quality of the fits were judged by the χ^2 values as well as by the weighted residuals of the fits.

Results

More than 14 series of time-resolved spectra, each with up to 15 fluorescence decays, were recorded varying the excitation wavelength, the excitation intensity and the sample pumping speed. Low pumping speed (≈ 10 ml/min) and a high laser intensity (≈ 5–10 mW/mm^2) together with a preillumination of the sample leads to closed RCs in the cells (quinone Q_A reduced, henceforth referred to as 'closed' RCs). Vice versa, high pumping speed (≈ 300 ml/min) and a low laser excitation intensity (≈ 1–2 mW/mm^2) keeps most RCs in the open state (henceforth referred to as 'open' RCs) [31]. Using an excitation wavelength of 720 nm the BChl-*c* pigment pool in the chlorosomes is excited selectively. At this excitation wavelength five lifetime

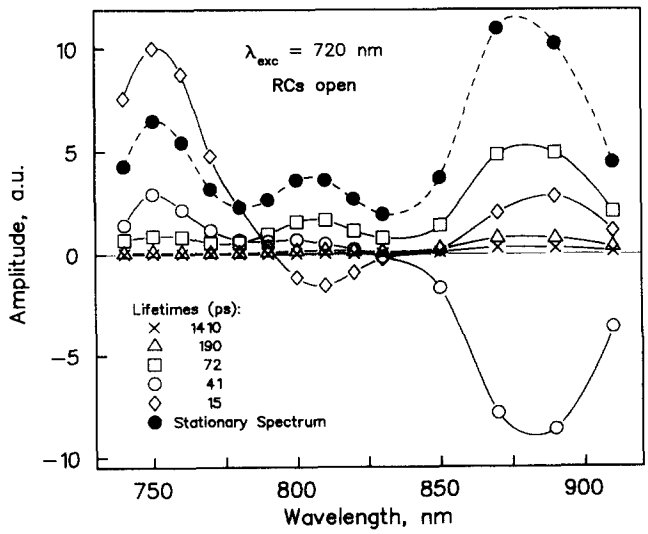


Fig. 1. DAS of living cells *C. aurantiacus* as obtained by global analysis of the fluorescence decays measured at 50°C with open reaction centers and an excitation wavelength of 720 nm. The dashed line indicates the steady state emission spectrum.

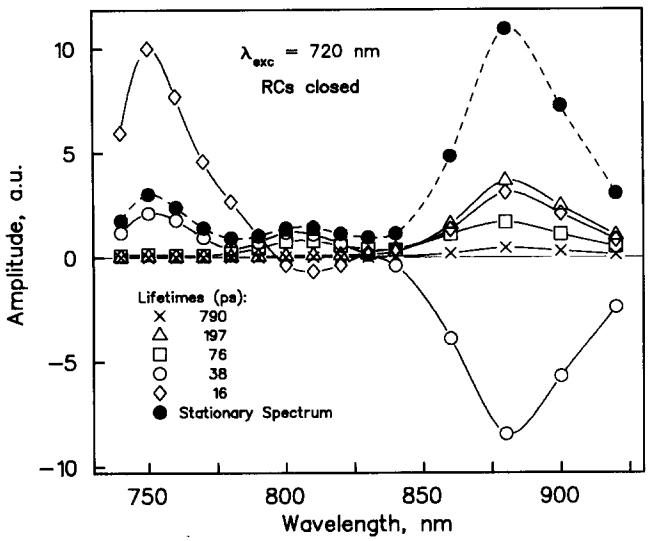


Fig. 2. DAS of living cells *C. aurantiacus* as obtained by global analysis of the fluorescence decays measured at 50°C with closed reaction centers and an excitation wavelength of 720 nm.

components were necessary for a good fit of the fluorescence decays independent of the photochemical redox state of the RCs in the cells. Figs. 1 and 2 represent the DAS for *C. aurantiacus* cells with RCs in the open and in the closed state, respectively. In both cases the lifetimes were very similar but the amplitude ratios differed strongly. With open RCs lifetimes of 15 ps, 41 ps, 72 ps, 190 ps and 1.4 ns were observed (Fig. 1) while the lifetimes with closed RCs were 16 ps, 38 ps, 76 ps, 197 ps and 790 ps (Fig. 2, see Table I for a collection of all lifetimes). The lifetime of the longest-lived component in the ns-range varied with the specific sample (c.f. Discussion section). Data analysis with only four components gave rise to strong systematic deviations in the weighted residuals and the components with the lifetimes of about 80 ps and 190 ps

TABLE I

Fluorescence lifetimes of living cells C. aurantiacus

Errors in the lifetimes due to the measurement and the analysis are less than 10% except for those of the four component analyses at 720 nm excitation.

λ_{exc} (nm)		τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	τ_4 (ps)	τ_5 (ns)	χ^2
720 [a]	(4 components)	15 [b]	38 [b]	102	–	1.14	1.385
	(5 components)	15 [b]	41 [b]	72	190	1.41	1.140
720 [c]	(4 components)	17 [b]	49 [b]	–	145	0.58	1.663
	(5 components)	16 [b]	38 [b]	76	197	0.79	1.126
790 [d]		–	35 [b]	67	188	1.10	1.078
815 [a]		–	33	83	386	1.56	1.161

[a] 50°C, reaction centers 'open'.
[b] Lifetime component shows positive and negative amplitudes.
[c] 50°C, reaction centers 'closed'.
[d] Room temperature, reaction centers partially 'closed'.

mixed into one component with a lifetime between 100 ps and 150 ps (c.f. Table I).

Results for open RCs

The DAS of the 15 ps component for cells with open RCs shows large positive amplitudes around 750 nm, turns negative in the wavelength range of the B_{792}-emission around 805 nm and is again positive in the emission range of the B_{866} pigments above 850 nm (Fig. 1). The DAS of the 41 ps component remains positive up to 830 nm but drops to strongly negative values in the B_{866}-emission region above 850 nm. The other components show only positive amplitudes over the whole detection wavelength range. The DAS of these components reveal one main maximum at about 880 nm and two lower amplitude maxima at 810 nm and 750 nm.

Results for closed RCs

With one notable exception, the lifetimes and the DAS for cells with closed RCs (Fig. 2) are essentially the same as for cells with open RCs (Fig. 1). The difference refers mainly to the amplitude ratio of the 76 ps component and the 197 ps component in the B_{866} emission (Fig. 2). In the fluorescence decays with open RCs the amplitude of the ≈ 190 ps component is about 6-times smaller than the amplitude of the 72 ps component (Fig. 1). Upon closing the RCs this amplitude ratio rises to a value of about two i.e., the amplitude of the 197 ps component exceeds the amplitude of the 76 ps component (Fig. 2). However, the sum of amplitudes in the B_{866}-emission wavelength range is zero in either case. This corresponds to the fact that the B_{866} pigment pool is not populated immediately after the excitation by the laser pulse as expected for 720 nm excitation. The inversion of the amplitude ratio of the two lifetime components leads to a change in the relative height of the maxima in the stationary emission spectra. These changes in the amplitude ratio are a consequence of the observed fluorescence induction ratio $F_{max}:F_0$ of about (2–3):1 as measured by the increase of the stationary fluorescence intensity in the B_{866}-emission range when switching the pump off.

Long excitation wavelengths

Using excitation wavelengths of 790 nm or 815 nm the fluorescence kinetics of living cells is reduced to four components only. A fifth component with a lifetime of 15 ps is not resolved with either 790 nm excitation or 815 nm excitation. With 790 nm excitation the lifetimes of the remaining components are about 35 ps, 67 ps, 188 ps and 1.1 ns, i.e., similar to the lifetimes found for 720 nm excitation. Their DAS also resemble those of the measurements with 720 nm excitation in the detection wavelength range above 800 nm (Fig. 3). Especially the 35 ps component shows again strong

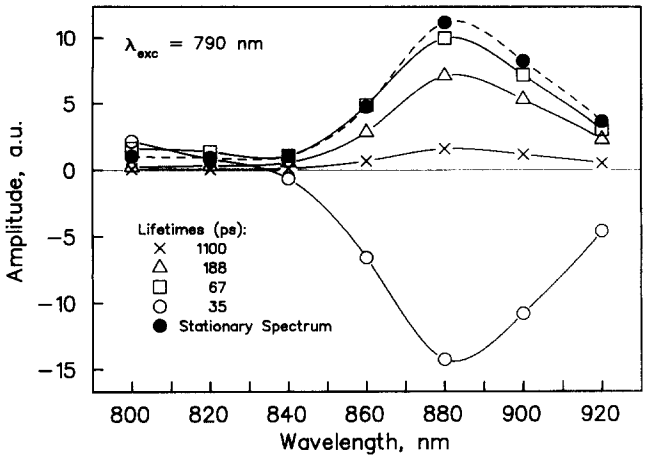


Fig. 3. DAS of living cells *C. aurantiacus* as obtained by global analysis of the fluorescence decays measured at an excitation wavelength of 790 nm and at room temperature. The reaction centers were partially closed (see text).

negative amplitudes in the B_{866}-emission range. However, the amplitude ratio of the 67 ps component and the 188 ps component is only about 1.2:1. This is due to the fact that these measurements were performed at room temperature. Under such conditions more RCs are closed in living cells of the thermophilic bacterium *C. aurantiacus* due to a kinetic limitation of Q_B diffusion [37,38] as compared to measurements at 50°C under the same excitation and pumping conditions. At an excitation wavelength of 815 nm the photosynthetic system is excited predominantly in the B_{806}-absorption band. In this case lifetimes of 33 ps, 83 ps, ≈ 390 ps and ≈ 1.6 ns were obtained. All four components reveal positive DAS only, which follow within the error limits the stationary emission spectra with a maximum

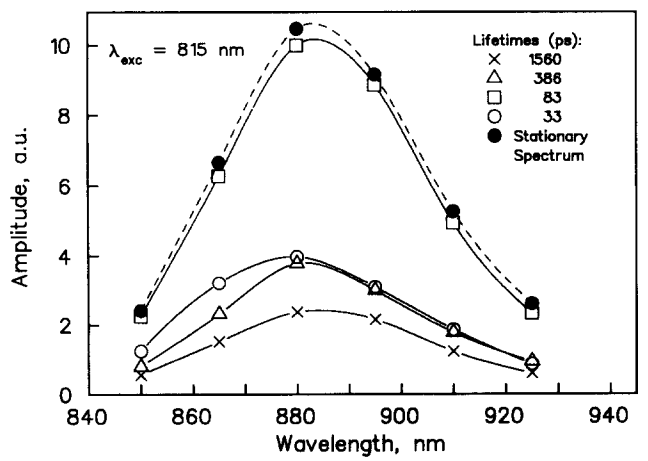


Fig. 4. DAS of living cells *C. aurantiacus* as obtained by global analysis of the fluorescence decays measured at an excitation wavelength of 815 nm and a temperature of 50°C with open reaction centers.

at about 880 nm (Fig. 4). The fluorescence kinetics are clearly dominated by the 83 ps component. The other components show only 25–40% of the amplitude of the 83 ps component. Note that also the amplitude of the 33 ps component is positive at this excitation wavelength. The fluorescence lifetimes of the living cells observed under the various conditions are compiled in Table I.

Discussion

Energy transfer processes

The kinetics of the chlorosomal components does not significantly depend on the redox state of the reaction center. Thus, for both open and closed RCs the 15 ps component (Figs. 1 and 2) with positive amplitudes around 750 nm and negative amplitudes above 795 nm provides direct evidence for an energy transfer process from the chlorosomal BChl-*c* pigment pools to the B_{792} pigment pool in the baseplate of the chlorosome as already demonstrated earlier [1]. Furthermore, the strong positive amplitude of the 15 ps component in the detection wavelength range of the B_{866}-emission contains important information about the arrangement of the pigment pools and will be discussed below. The second chlorosomal energy transfer process occurs with a time constant of 35–40 ps and reflects the transfer from the B_{792} pigment pool in the baseplate to the B_{806} chromophores in the cytoplasmic membrane. This is proven by the characteristic positive and negative DAS of the corresponding lifetime component at the three different excitation wavelengths (Figs. 1 to 4). An energy transfer component with a time constant of 35–40 ps in the fluorescence kinetics of living cells has already been reported before by Mimuro et al. [25] and ourselves [1] for an excitation wavelength of 715–720 nm. For an excitation wavelength of 815 nm the 35–40 ps component is no longer a rise term in the B_{866}-fluorescence but turns into a decay term (Fig. 4). The observation of the 35–40 ps component as a decay term with positive amplitudes in the B_{866}-emission wavelength range is due to energy back transfer to the B_{792} pigment pool. This can easily be verified by a kinetic computer simulation that calculates the DAS for an excitation wavelength of 790 nm and 815 nm using the same transfer rate constants (e.g. $k_{B792 \rightarrow B806-866} = 24\ ns^{-1}$ and $k_{B806-866 \rightarrow B792} = 2\ ns^{-1}$) for the energy transfer processes in either case. The qualitative behavior of the 35–40 ps component equilibration component does not depend substantially upon the specific rate constants chosen in the model. Even if we take into account a comparatively fast 5 ps equilibration process within the $B_{806-866}$ complex the amplitude of the 35–40 ps component in the B_{866}-emission remains positive for 815 nm excitation. Thus the positive amplitude of the 35–40 ps component at about 880

nm does not reflect the trapping process in living cells with open RCs as proposed by Causgrove et al. [27].

The energy transfer processes in the isolated $B_{806-866}$ antenna complex, the GEF-chlorosomes [33,39,40] and the SDGC-chlorosomes have already been reported [16,29,30]. In two of these complexes, i.e., GEF-chlorosomes and the $B_{806-866}$ complex, relaxations are much faster than the ones discussed above and have not been resolved in the kinetics of living cells. The reasons for this discrepancy between isolated complexes and intact cells is most likely the complexity of the fluorescence kinetics of living cells. For isolated GEF-chlorosomes we reported a ≈ 5 ps relaxation process between BChl-*c* pigment pools or exciton states when exciting at ≈ 720 nm [29]. The presence of such an ultrafast component also in SDGC-chlorosomes was confirmed by transient absorption data [41]. In intact cells the kinetics is even more complex and thus we can not expect to resolve this ultrafast component. It is not quite clear whether two types of BChl-*c*-aggregates or exciton state relaxation are responsible for this kinetic component. From studies of artificial BChl-*c*-aggregates [42,43] we have concluded that two different BChl-*c* pigment pools (two different aggregate types) are possibly present in chlorosomes. According to this model, the kinetic component with a lifetime of about 5 ps describes the energy transfer between the pigment pools BChl-c_I and BChl-c_{II} [29]. The shape of the DAS of this kinetic component varies somewhat depending upon the specific batch of the isolated chlorosomes (for details see Ref. 35). An analogous dependence was observed in the circular dichroism spectra [34]. The circular dichroism spectra of different chlorosome batches and the corresponding membrane samples prepared in parallel were best fitted by a sum of two basic spectra with varying weighting coefficients [34]. The assumption of two aggregate types which are synthesized by the organism with varying ratio could explain both the DAS and the circular dichroism spectra. Nevertheless, the possibility cannot be excluded that relaxation processes of excitonically coupled excited states are contributing to the complex kinetics.

The energy transfer between the chlorosomal BChl-*c* pigment pools and the B_{792} chromophores in the baseplate was first observed in isolated SDGC-chlorosomes [29,30]. The equilibration time constant for this energy transfer process was 11–16 ps and the average lifetime appears to be somewhat shorter than in intact cells. This may, however, be due to somewhat differing chlorosome sizes in these measurements [44], which may not be avoided using batch cultures. Another, and perhaps better explanation, for this discrepancy might consist in the fact that the measured times are actually equilibration times and not simply energy transfer times. Intact cells are more complex coupled kinetic systems and therefore apparent equilibration times will differ between cells and isolated chlorosomes even if the individual rate constant for a particular step is the same.

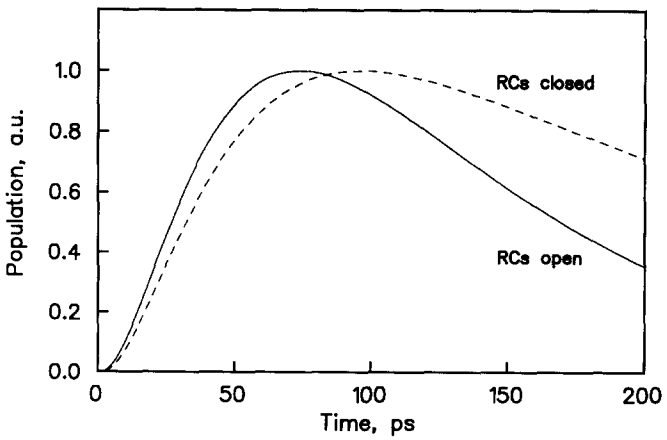


Fig. 5. Excited state population of the B_{866} pigment pool as a function of time calculated on the basis of the results given in Fig. 1 and Fig. 2 for open (full line) and closed reaction centers (dashed line). It is assumed that the cells were excited via the chlorosomal chromophores. Both curves are normalized to one in the maximum.

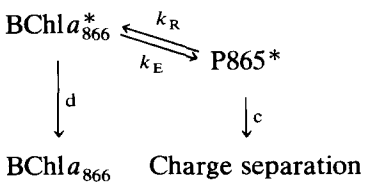
We will now return to the phenomenon of the strong positive amplitudes of the 15–16 ps component in the B_{866}-emission wavelength range (Fig. 1 and Fig. 2). This feature indicates a sequential energy transfer scheme where most of the energy is transferred from the chlorosomal BChl-*c* pigments to the BChl-*a* pigments in the cytoplasmic membrane via the B_{792} pigment pool [1]. The 35–40 ps rise term together with the 15–16 ps decay term in the B_{866}-fluorescence means that the excited state population of B_{866} increases with a time constant of about 35–40 ps after a delay of about 15–16 ps. This requires that the B_{792} pigment pool is excited first before the energy transfer to B_{806} and consequently to B_{866} chromophores will proceed. The excited state population of the B_{866} pigments as a function of time was calculated for an excitation wavelength of 720 nm (chlorosome excitation) on the basis of the measured fluorescence decay kinetics (Fig. 1 and Fig. 2) and is shown in Fig. 5. A purely sequential transfer model with backtransfer is in contrast to the proposal of Blankenship et al. [26,45] who claimed the possibility of a direct energy transfer from the chlorosomal BChl-*c* to the cytoplasmic BChl-*a*-chromophores. We can support the purely sequential energy transfer scheme on the basis of a comparison of simulated DAS and experimental ones. In these simulations rate constants above $(200\ \text{ps})^{-1}$ for a supposed direct energy transfer BChl-*c* $\rightarrow B_{806}$ lead to positive amplitudes of the 15–16 ps component in the B_{866}-fluorescence which are much lower than observed experimentally. For rate constants higher than $(55\ \text{ps})^{-1}$ these amplitudes would be negative, which would be even more in disagreement with the experimental data (Figs.

1 and 2). However, some quenching mechanism occurring within the chlorosomes may lead to higher amplitudes of the shortest-lived components in the BChl-*c*-fluorescence. This effect could be erroneously misinterpreted as a direct energy transfer.

Energy trapping processes

The energy transfer processes in the antenna system of *C. aurantiacus* are basically independent from the photochemical redox state of the RCs. In contrast, the energy trapping processes turn out to be strongly affected if the RCs change from the open to the closed state (Figs. 1 and 2). The energy trapping process can be monitored by the fluorescence decay of the longest wavelength pigment pool, i.e., the B_{866} complex. In living cells the excited state of the antenna system is depopulated predominantly with a time constant of 70–90 ps if the RCs are open (Fig. 1) and with a time constant of 180–200 ps if the RCs are closed (Fig. 2). This observation gives a first indication that the trapping process of the excitation energy is essentially determined by the charge separation rate in the RC and not by the energy transfer from the B_{866} chromophores to the special pair in the RC. Lifetime components of 245 ps and 200 ps have also been found by several groups in time-resolved fluorescence and absorption measurements [25,27,46]. Causgrove et al. [27] also suggested that this lifetime is related to the energy trapping process in cells with closed RCs.

The assignment of the decay components with lifetimes of 70–90 ps and 180–200 ps to the energy trapping process in cells with RCs open on the one hand and closed ones on the other hand is also supported by our previous results with isolated RCs of *C. aurantiacus* [31]. These data revealed a charge separation time of 6–7 ps in the open state ($P^*HQ \rightarrow P^+H^-Q$) and of about 18 ps in the closed state ($P^*HQ^- \rightarrow P^+H^-Q^-$). The dependence of the energy trapping times in living cells on the charge separation times in isolated RCs allows a calculation of the rate constants for the energy transfer processes between the B_{866} chromophores and the special pair in a simple two-level-model. This model is shown in Scheme I and assumes a deactivation rate constant of $d = 1\ ns^{-1}$ for the excited states of the B_{866} chromophores [16] and rate constants of $c_0 = (6.7\ ps)^{-1}$ and $c_{cl} = (18\ ps)^{-1}$ for the charge separation to the bacteriopheophytin [31] with RCs open and closed, respectively.



Scheme I.

TABLE II

Rate constants for the energy trapping process by the reaction center

The rate constants k_E for the energy transfer to the special pair and k_R for the back transfer to the B_{866} chromophores were calculated with Eqn. 1 on the basis of the model described in the text. The time constants for the energy trapping in living cells with open (τ_0) and closed reaction centers (τ_{cl}) are input parameters.

τ_0 (ps)	τ_{cl} (ps)	k_E (ns^{-1})	k_R (ns^{-1})	k_R/k_E
80	180	98	1040	10.6
90	180	33	320	9.6
90	190	48	520	10.8

The charge recombination to the excited state P* is slower than $(5\ ns)^{-1}$ [31] and can be neglected in the model. The rate constants for the energy transfer processes from B_{866} to the special pair (k_E) and back into the B_{866} antenna (k_R) should be independent of the redox state of the quinone. Thus they can be calculated using a set of two linear equations (Eqn. 1) for the trapping time in cells with RCs open (τ_0) and closed (τ_{cl}).

$$\frac{k_E}{(1/\tau_0 - d)} + \frac{k_R}{(1/\tau_0 - c_0)} = 1$$
$$\frac{k_E}{(1/\tau_{cl} - d)} + \frac{k_R}{(1/\tau_{cl} - c_{cl})} = 1 \qquad (1)$$

Some results for these rate constants as well as their ratio k_R/k_E are presented in Table II. It can be seen that the absolute values of the rate constants vary strongly with the specific combination of the trapping times τ_0 and τ_{cl}. However, the ratio of these rate constants k_R/k_E remains constant in the range of 10–11. If this value is divided by the Boltzmann factor for the energy difference between the excited states of the B_{866} and the special pair P we can obtain the number of B_{866} chromophores which are coupled energetically to the special pair of the RC. The energy difference may be calculated to a good approximation by the average of the absorption and emission maxima for the B_{866} chromophores on the one hand and the special pair on the other hand. This difference is about 90 cm^{-1}. At a temperature of 50°C the Boltzmann factor for this energy difference is 0.67 and thus it follows a number of 15–16 B_{866} chromophores per RC. Such a value is basically in agreement with the value of 20–25 derived by exciton annihilation experiments [46] if we take into consideration the differences in the two methods used. Our value is also in good agreement with biochemical estimates of 6 $B_{806-866}$ complexes per RC [43].

For some combinations of the trapping times the rate constants calculated using Eqn. 1 will be negative. In this case the effect of closing the RC on the energy trapping time in living cells would be higher than on

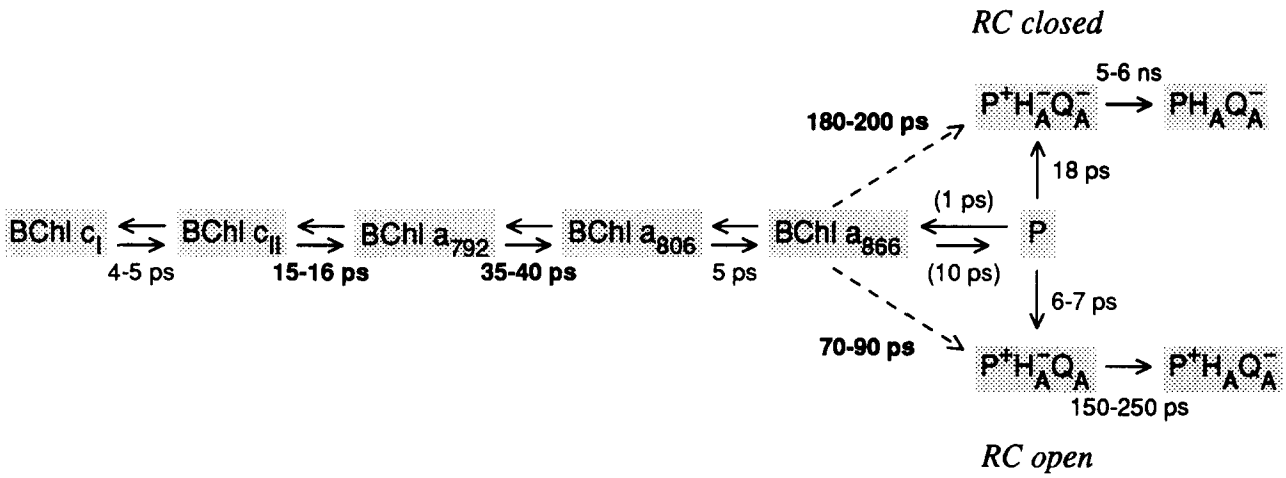


Fig. 6. Kinetic scheme summarizing the results of intact cells (lifetimes of this work in bold letters) as well as those from our previous work on the isolated complexes (plain letters) [16,29–32,35]. The lifetimes indicated with broken arrows are overall trapping times (measured lifetimes). Note that the energy transfer times from B_{866} to the RC and vice versa are calculated ones. Only the ratio of the lifetimes is determined precisely from Boltzmann equilibrium, the absolute lifetimes may vary to some extent (c.f. Table II).

the charge separation time in isolated RCs without antenna system. This situation is physically not reasonable. From Table II we can estimate the order of magnitude of the rate constants for the energy transfer from the B_{866} to the special pair and back into the antenna to be $\approx (10\ \text{ps})^{-1}$ and $\approx (1\ \text{ps})^{-1}$, respectively.

The fluorescence kinetics of living cells contains two additional decay components with longer lifetimes of about 400 ps and 1–1.5 ns. The components can be assigned in one part to the relaxation of $B_{806-866}$ complexes which are not coupled to a RC [16] and for another part to the secondary electron transfer step from the bacteriopheophytin to the quinone in the RC. The origin of the nanosecond components is also due to charge recombination processes in the RC [31]. The fluorescence lifetime measurements of the isolated RCs from *C. aurantiacus* reveal lifetimes of about 150–250 ps for the secondary electron transfer to the quinone and about 5–6 ns for the charge recombination [31]. The difference may occur due to a short fitting window in this analysis concentrating on the short-living kinetic components.

In a recent paper, Mimuro [28] also studied energy-transfer and trapping proccesses in intact cells of *C. aurantiacus*. Despite the different analysis method the general energy-transfer scheme resulting from that work is quite similar to the one proposed here. Nevertheless, there are also some important differences. First, the energy-transfer time BChl-*c* $\rightarrow B_{792}$ is shorter than found in this work (7 ps as compared to $\approx$ 15 ps). Second, the $B_{806} \rightarrow B_{866}$ energy transfer time was estimated to be < 1 ps by Mimuro [28]. We measured this time to be 5 ps in the isolated complex [16]. A further, and perhaps the most important, difference is that we were not able, neither in the isolated $B_{806-866}$ complex [16] nor in whole cells, to detect any separate long-wavelength pigment pool present in the $B_{806-866}$ complex. We did not observe any different kinetics above $\approx$ 890 nm than at lower wavelength for B_{866}-fluorescence. We therefore conclude that, according to our data, such an inhomogeneity is not present in whole cells or the isolated complex at the temperatures we used for measurements. This is reminiscent of a similar lack of kinetic inhomogeneity in data we obtained recently from *Rb. capsulatus* chromatophores [47].

Conclusions

Based on the results of all lifetime measurements with the isolated complexes together with those for the living cells a complete scheme for the primary photophysical and photochemical processes of the photosynthetic system of *C. aurantiacus* can be drawn. This is shown in Fig. 6 and contains both the energy transfer processes in the antenna system as well as the electron transfer processes in open and closed RCs.

The energy transfer chain represents a linear cascade of energy transfer steps from the shorter wavelength absorbing chromophores in the chlorosome to the longer wavelength absorbing chromophores in the cytoplasmic membrane. However, the equilibration times of 4–5 ps, 15–16 ps, 35–40 ps and $\approx$ 5 ps indicate a bottleneck of the energy transfer between the B_{792} complex in the baseplate of the chlorosome and the $B_{806-866}$ complexes in the cytoplasmic membrane. One possible explanation for this relatively slow energy transfer might be given by an evolutionary aspect. It is believed that the chlorosomes were built up later in the phylogenetic development of *C. aurantiacus* by lateral gene transfer from other photosynthetic organisms and were thus linked later to the $B_{806-866}$

complex [48–50]. Also the occurrence of a species closely related to *Chloroflexus* named *Heliothrix oregonensis* but not containing chlorosomes may point to this possibility [51]. Even with open RCs the energy transfer processes are much faster than the trapping times by the charge separation in the RC. Thus the fluorescence decay kinetics of living cells is trap-limited by the RC and not diffusion-limited by the energy transfer processes. Generally the excitation energy visits the special pair about five to ten times until the charge separation to the bacteriopheophytin takes place. This demonstrates the necessity of a very fast primary electron transfer step with the lifetime of about 6–7 ps. In this way the whole photosynthetic system appears to be optimized in a comprehensive manner.

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