Volume 250, number 2, 503–508 FEB 07312 July 1989

Characterization of excitation energy trapping in photosynthetic purple bacteria at 77 K

H. Bergström, R. van Grondelle* and V. Sundström

Department of Physical Chemistry, University of Umeå, Umeå, Sweden and **Biophysics Department, Physics Laboratory, Free University of Amsterdam, Amsterdam, The Netherlands*

Received 10 May 1989

We have studied the energy-transfer dynamics in chromatophores of *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* at 77 K, with functional charge separation. Using low-intensity picosecond absorption recovery, we determined that transfer between the energetically low-lying antenna component BChl896 and the special pair of the reaction center occurs with a time constant of 37 ps in *Rb. sphaeroides* and 75 ps in *R. rubrum*. Assuming that a Förster energy-transfer mechanism applies to the process, this allows us to estimate the distance between BChl896 in the B875 complex and the special pair P870 in the reaction center to range between 26 and 39 Å in *Rb. sphaeroides*. Such a distance indicates that the BChl896 pigment and the special pair of the reaction center are at the minimum separation allowed by the size and shape of the reaction center and the light-harvesting polypeptides.

Energy transfer; Picosecond absorption spectroscopy; Bacteriochlorophyll; Reaction center; Light-harvesting antenna

1. INTRODUCTION

The dynamics of energy transfer and trapping in photosynthetic purple bacteria have recently been extensively studied by means of picosecond absorption [1,2] and fluorescence measurements [3,4]. These experiments have clearly shown that the bacteriochlorophyll light-harvesting antenna is inhomogeneous and consists of several different pigment pools forming an energetic downhill arrangement for energy transfer to the special pair of bacteriochlorophylls (called P870) of the reaction center (RC). For *R. rubrum* and *Rb. sphaeroides*, which we have studied in this report, the main antenna consists of one (B880) and two (B800–850, B875) pigment-protein complexes, respectively. In both species there also exists an additional minor red-shifted component termed B896 [1,2,5,6].

Steady-state fluorescence depolarization measurements at 4 K [5], picosecond absorption measurements at room temperature [1] and 77 K [2], as well as spectroscopic work with photosynthetic mutants [6], have shown that B896 is an integral part of the B875/B880 core antenna. Its spectral properties may arise as a consequence of pigment-pigment or pigment-protein interactions, and the function of B896 is probably to concentrate the excitation energy into the vicinity of the reaction center, thereby increasing the probability of trapping [7].

As a result of picosecond absorption measurements on chromatophores [1] and isolated pigment-protein complexes [8,9] of purple bacteria, the energy transfer at room temperature can be characterized by the following sequences and time constants seen when the RC is able to perform light-driven charge separation [1,8,9], i.e. the RCs are 'open'.

Rb. sphaeroides

$$\text{BChl800} \xrightarrow{\approx 0.5\ \text{ps}} \text{BChl850} \underset{}{\overset{35\text{–}40\ \text{ps}}{\rightleftharpoons}}$$

$$\text{BChl875} \underset{}{\overset{60\ \text{ps}}{\rightleftharpoons}} \underline{(\text{BChl896})}\ \text{RC(open)}$$

Correspondence address: H. Bergström, Department of Physical Chemistry, University of Umeå, Umeå, Sweden

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

R. rubrum

$$\text{BChl880} \underset{}{\overset{60\ \text{ps}}{\rightleftharpoons}} \underline{(\text{BChl896})}\ \text{RC(open)}$$

The energy differences between adjacent pigment pools in these sequences (except BChl800/850) at room temperature are small compared to kT. Energy equilibrium is therfore established between the pools shortly after excitation, and the measured excited-state decays will reflect this fact. As a result of such equilibration processes the BChl875 (/BChl880) $\rightleftharpoons$ BChl896 energy-transfer step cannot be distinguished from the BChl896 $\rightleftharpoons$ RC trapping process at room temperature, and only an average trapping time constant of about 60 ps is observed [1].

When the energy-transfer kinetics are measured at 77 K, all transfer steps are effectively irreversible and the individual energy-transfer processes can be resolved [2,10]. Picosecond absorption measurements have allowed us to characterize the energy-transfer process in purple bacteria at 77 K, with the primary donor (P870) of the reaction center in its oxidized state ($P870^+$). Under these conditions, no charge separation is possible, but the excitation energy is still quenched relatively efficiently by the oxidized primary donor, i.e. the RCs are not able to perform light-driven charge separation and are 'closed'.

For *Rb. sphaeroides* the measured kinetics was interpreted as the following sequence of events [2,10]:

Rb. sphaeroides

$$\text{BChl800} \xrightarrow{1\text{–}2\ \text{ps}} \text{BChl850} \xrightarrow{40\ \text{ps}} \text{BChl870} \overset{10\text{–}15\ \text{ps}}{\rightleftharpoons}$$

$$\text{BChl875} \xrightarrow{20\ \text{ps}} \text{BChl896} \xrightarrow{190\ \text{ps}} \text{RC(closed)}$$

For *R. rubrum*, which lacks the B800–850 pigment, the scheme becomes:

$$\text{BChl880} \xrightarrow{20\ \text{ps}} \text{BChl896} \xrightarrow{150\ \text{ps}} \text{RC(closed)}$$

It is of considerable interest to obtain the rate of the trapping step BChl896 $\longrightarrow$ RC with the reaction centers in the active, open, state, and able to effect charge separation, since this would provide insights into the pigment organization in the immediate surroundings of the reaction center complex. Such an experiment would also clearly establish the time scales of energy transfer and trapping in the primary reactions of photosynthesis.

Here, we have studied the trapping of excitation energy by the reaction center, in chromatophores of *Rb. sphaeroides* and *R. rubrum* at 77 K. The experimental conditions (low temperature and continuous illumination of the sample) made it impossible to maintain a fully active electron-transport chain, but nevertheless the primary step of charge separation from the special pair to the primary electron acceptor bacteriopheophytin (Bph), P870 Bph $\longrightarrow$ $P870^+$ Bph^-, was operating and in the context of our experiments the RCs are open. In the present experiments, the minor antenna component B896 was directly excited by an infrared picosecond pulse and the trapping of excitation energy was monitored by picosecond absorption spectroscopy [1]. The results show that the final trapping step is relatively slow; the time constant associated with this process was measured to be 37 ± 5 ps in *Rb. sphaeroides* and almost a factor of two longer in *R. rubrum*. Assuming that the Förster mechanism of energy transfer applies we estimate an average center-to-center distance between BChl896 and P870 of about 3 nm in *Rb. sphaeroides*.

2. MATERIALS AND METHODS

In order to resolve the BChl896 $\longrightarrow$ P870 trapping step, the equilibrium situation established when there is energy back-transfer must be avoided. Consequently, the experiments were performed at 77 K. However, since the picosecond laser used delivers a continuous train of picosecond pulses with a repetition rate of 80–800 kHz, and the frozen sample cannot be flowed, maintaining the reaction centers in their fully active state is a difficult task. This forced us to use experimental conditions where only the primary charge separation step from the special pair P870 to the primary acceptor bacteriopheophytin (Bph) is active. Experimentally, this was realized by pre-reducing the secondary electron acceptor (Q_A) with sodium dithionite (10 mM in *Rb. sphaeroides* and 50 mM in *R. rubrum*) in a buffered solution (50 mM Tricine) with a 1 : 2 water/glycerol ratio, at pH 8, and under anaerobic conditions. Immediately after preparation the samples were cooled to 77 K where they form a transparent glass. Excitation of P870 then leads to primary charge separation and formation of the state $P870^+Bph^-Q_A^-$. The recombination of this state occurs with a time constant of about 20–25 ns and yields either the original ground state or the reaction center bacteriochlorophyll triplet state [11]. The latter is rapidly converted into a reaction center carotenoid triplet

state [11]. The carotenoid triplet has a lifetime of a few microseconds [11]. At relatively low pulse intensities the reaction centers can thus be maintained in the active state, even during illumination with a pulse train having a pulse repetition rate of 80–800 kHz.

Picosecond absorption recovery kinetics were measured using the technique described in [1]. In order to establish the open reaction center state and avoid non-linear effects, it is necessary to use a light intensity as low as possible when measuring the kinetics. Maximum average and peak pulse intensities of 4.2×10^{19} photons·s^{-1}·cm^{-2} and 5.1×10^{13} photons·cm^{-2}·$pulse^{-1}$, respectively, were employed in the present measurements, and the kinetics remained unchanged when the intensity was decreased 10-fold.

3. RESULTS AND DISCUSSION

For excitation wavelengths longer than 905 nm the B896 pigment is selectively excited in *Rb. sphaeroides* [2], and if the same analyzing wavelength is used, energy transfer out of B896 is manifested by recovery of the B896 bleaching and formation of a constant bleaching due to $P870^+Bph^-$. The difference absorption (ΔA) spectrum of $P870^+Bph^-$ is known to possess a bleaching band in the wavelength region 870–910 nm, with a maximum around 890 nm [12]. The kinetic curve of fig.1 obtained on *Rb. sphaeroides* chromatophores at 905 nm, shows that the bleaching due to the initially formed BChl896* decays with a time constant of 37 ps into a long-lived bleaching of low intensity due to $P870^+Bph^-$. A similar appearance of the kinetics is observed at other wavelengths above 900 nm. At shorter wavelengths the excited state of B896 is observed as an absorption increase [2], and the same time constant for the BChl896* decay is obtained.

The characteristic shape of the ΔA spectrum of $P870^+Bph^-$ around 800 nm [12] is used to confirm further the identity of the long-lived bleaching observed at 905 nm. The ΔA spectrum of isolated reaction centers displays maximum absorption at 790 nm and maximum bleaching at about 810 nm [12]. Absorption recovery measurements performed at these two wavelengths are in full agreement with the assignment of the constant signal formed after the decay of BChl896*, to the charge-separated radical pair $P870^+Bph^-$. This is illustrated by the other kinetic curves displayed in fig.1. The formation of a long-lived bleaching at 810 nm and long-lived absorption at 790 nm proves that the $P870^+Bph^-$ state is developed. The

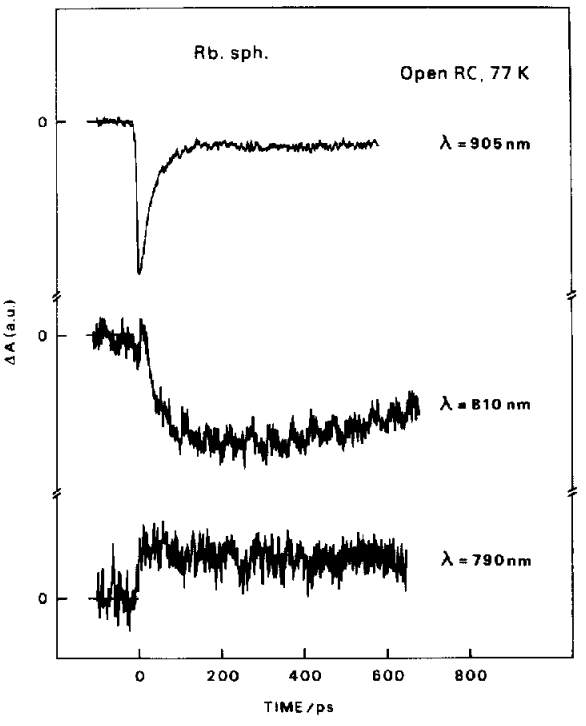

Fig.1. Absorption recovery kinetics of *Rb. sphaeroides* chromatophores at 77 K measured at three different wavelengths. Open RC refers to the initial state of the reaction center, with the primary donor P870 reduced, the primary acceptor Bph oxidized and the secondary acceptor Q_A reduced, i.e. P870 Bph Q_A^-. This state is capable of trapping excitation energy from the antenna by formation of the charge-separated state, $P870^+Bph^-Q_A^-$. The long-lived (≫1 ns) kinetic component observed in all traces reflects the presence of this species.

small difference in extinction coefficients between antenna BChl and $P870^+Bph^-$ at these wavelengths makes the task of obtaining a time constant for the trapping from these traces a difficult one.

When the experiments described above were repeated for *R. rubrum*, a considerably longer time constant of about 75 ps for the final trapping step at 77 K was obtained as is shown in fig.2. Kinetics measured as a function of temperature show that the trapping time constant for *R. rubrum* increases from about 40 ps at 100 K to 75 ps at 77 K observed here [13], whereas for *Rb. sphaeroides* it remains constant (37 ps) over the entire temperature interval of 177–77 K. Previous measurements of the temperature dependence of the fluorescence quantum yield [14] have shown that the fluore-

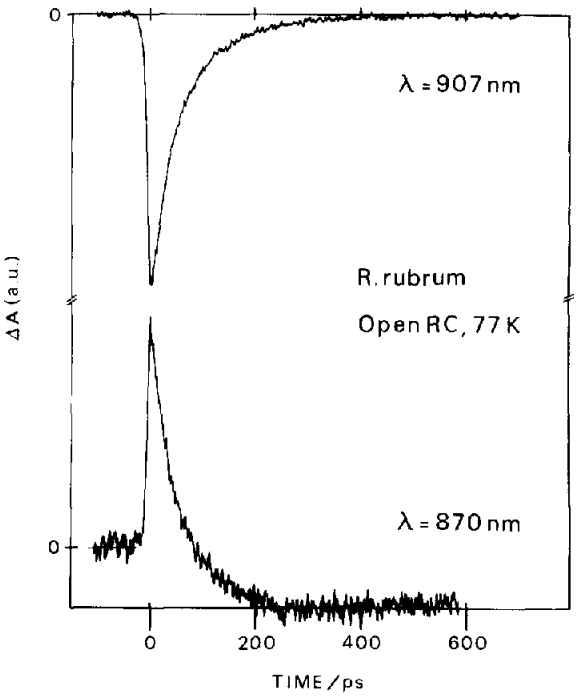

Fig.2. Absorption recovery kinetics of *R. rubrum* at 77 K, measured at two different wavelengths. Open RC has the same meaning as in fig.1.

scence quantum yield of both *R. rubrum* and *Rb. sphaeroides* starts rising at about 80 K when the temperature is lowered. This rise is however substantially sharper in *R. rubrum*. We suggest that the longer trapping time at 77 K observed for *R. rubrum* is a result of a temperature-induced spectral narrowing and/or a spectral shift, affecting the spectral overlap and energy-transfer efficiency; at physiological temperatures the rate of trapping is identical in the two species.

With the present measurement of the energy trapping step in purple bacteria, the energy flow through the antenna system of *Rb. sphaeroides* at 77 K, with primary charge separation operational, is characterized by the following scheme and time constants:

$$\text{BChl800} \xrightarrow{1\text{–}2\ \text{ps}} \text{BChl850} \xrightarrow{40\ \text{ps}} \text{BChl870} \rightleftharpoons$$

$$\text{BChl875} \xrightarrow{20\ \text{ps}} \text{BChl896} \xrightarrow{35\text{–}40\ \text{ps}} \text{RC(open)}$$

In the case of *R. rubrum* only the last two steps are relevant,

$$\text{BChl880} \xrightarrow{20\ \text{ps}} \text{BChl896} \xrightarrow{75\ \text{ps}} \text{RC(open)}$$

From the schemes above it is evident that the final trapping step occurs with a rate typical of other inter-complex energy-transfer processes in purple bacteria. The time constant of this step is of the same order of magnitude as the total trapping time observed at room temperature [1]. This result demonstrates that the final trapping step contributes substantially to the overall trapping time. One may speculate as to how well this agrees with the known high overall efficiency of energy transfer in these organisms. It appears that the minor B896 antenna component is a necessary feature for attaining the observed efficiency. With this low-energy antenna component, consisting of only a few BChl molecules per reaction center [6], the excitation energy is concentrated into the vicinity of the reaction center, which makes trapping more probable although it is fairly slow.

Assuming weak Förster coupling between BChl896 and P870, the distance between the chromophores can be estimated from the Förster relation for energy transfer [15,16]:

$$k_{ET} = k_F\left(\frac{R_0}{R}\right)^6$$

where k_F is the radiative lifetime; and

$$(R_0)^6 = \frac{9\ln 10\,\kappa^2}{128\pi^5 n^4 N_A}\,\frac{\int f(\lambda)\epsilon(\lambda)\lambda^2 d\lambda}{\int \frac{f(\lambda)}{\lambda^2} d\lambda}$$

In evaluating the integral in the numerator of the expression for R_0 we encounter a problem; the absolute value of the extinction coefficient of P870 as a function of wavelength, $\epsilon(\lambda)$, is not known at 77 K. Therefore, we estimate $\epsilon(\lambda)$ by combining an absorption spectrum of the isolated reaction center at 77 K [12] with information about the value of ϵ at 295 K [17] and data on the variation of ϵ when T is varied [18]. The steady-state fluorescence spectrum of the isolated B875(-896) complex at 77 K [19] gives $f(\lambda)$, and $k_F = 5.6 \times 10^7\ s^{-1}$ is obtained from the literature [20]. Since the solvent is mostly glycerol we have used $n = 1.5$ as the refractive in-

dex [21]. The rate of energy transfer is $k_{ET} = (37 \times 10^{-12}\ s)^{-1} - (650 \times 10^{-12}\ s)^{-1}$ where 650×10^{-12} is the excited-state lifetime of BChl896 in the absence of trapping as measured in the isolated B875 complex at 77 K [22].

When the steady-state fluorescence spectrum of the B875/896 complex is used to obtain $f(\lambda)$ of the energy donor (B896) for the calculation of R_0, there is also a small contribution from B875 fluorescence. To correct for this contribution we have repeated the same calculations with the deconvoluted emission spectrum of BChl896 deduced from time-resolved emission measurements by Shimada et al. [23]. This emission spectrum is approximated as a Gaussian band with λ_{max} at 925 nm and a full-width at half-maximum of 27.8 nm. This yields an R_0 value which is about 25% less than that obtained using the total steady-state emission spectrum.

This leaves us with the question as to the value of the orientation factor $\varkappa^2$ in this pigment system. From the crystal structure of the reaction center [24] and measurements of linear dichroism [25], it is clear that P870 has the transition moment oriented parallel to the membrane plane. BChl896 is assumed to have the Q_y transition parallel to the membrane plane [5], and judging from measurements of the anisotropy decay at 77 K [2,22], which suggest transfer among differently oriented BChl896 molecules, we assume, for simplicity, the BChl896 transition dipole moments to have a circularly degenerate orientation in the plane. This allows us to calculate a value of 1.2 for $\varkappa^2$. Using this value of $\varkappa^2$ as an upper limit and the value $\varkappa^2 = 2/3$, in the case of a completely random orientation of the transition dipoles, as a lower limit, we calculate the distance, R, between BChl896 and P870 to be R = 35–39 Å (R_0 = 97–107 Å) when using the total emission spectrum, and R = 26–31 Å (R_0 = 73–86 Å) using the deconvoluted BChl896 spectrum.

Considering the size of the reaction center in the membrane plane, an ellipse with minor and major radii of at least 20 and 35 Å [26], and noting the central position of P870 within the reaction center polypeptides [24], it is clear that a distance between BChl896 and P870 of 26–39 Å implies that the B875 complex with the B896 pigment is situated at a distance from the special pair which is determined by the physical dimensions of the reaction center polypeptides. In view of the fact that the shorter estimate for R is probably more likely to be correct, we predict that the B875(-896) antenna interacts with the reaction center polypeptides, and that excitation energy enters the reaction center, along the short axis of the complex.

REFERENCES

[1] Sundström, V., Van Grondelle, R., Bergström, H., Åkesson, E. and Gillbro, T. (1986) Biochim. Biophys. Acta 851, 431–446.

[2] Van Grondelle, R., Bergström, H., Sundström, V. and Gillbro, T. (1987) Biochim. Biophys. Acta 895, 313–326.

[3] Godik, V.I., Timpmann, K.E., Freiberg, A.M., Borisov, A.Yu. and Rebane, K.K. (1986) Dokl. Akad. Nauk SSSR 289, 714–718.

[4] Godik, V.I., Freiberg, A.M., Timpmann, K.E., Borisov, A.Yu. and Rebane, K.K. (1987) in: Progress in Photosynthesis Research (Biggins, I. ed.) vol.1, pp.41–44, Martinus Nijhoff, Dordrecht.

[5] Kramer, H.J.M., Pennoyer, J.D., Van Grondelle, R., Westerhuis, W.H.J., Niedermann, R.A. and Amesz, J. (1984) Biochim. Biophys. Acta 767, 335–344.

[6] Van Dorssen, R.J., Hunter, C.N., Van Grondelle, R., Korenhof, A.H. and Amesz, J. (1988) Biochim. Biophys. Acta 932, 179–188.

[7] Van Grondelle, R., Bergström, H., Sundström, V., Van Dorssen, R.J., Vos, M. and Hunter, C.N. (1987) in: Photosynthetic Light-Harvesting Systems, Organization and Function (Scheer, H. and Schneider, S. eds) pp.519–530, De Gruyter, Berlin.

[8] Bergström, H., Sundström, V., Van Grondelle, R., Gillbro, T. and Cogdell, R. (1988) Biochim. Biophys. Acta 936, 90–98.

[9] Bergström, H., Sundström, V., Van Grondelle, R., Åkesson, E. and Gillbro, T. (1986) Biochim. Biophys. Acta 852, 279–287.

[10] Hunter, C.N., Bergström, H., Van Grondelle, R. and Sundström, V. (1989) in preparation.

[11] Parson, W.W. and Monger, T.G. (1976) in: Chlorophyll-Proteins, Reaction Centers, and Photosynthetic Membranes (Olsen, J.M. and Hind, G. eds) vol.28, pp.195–212, Brookhaven Symposia in Biology.

[12] Shuvalov, V.A., Shkuropatov, A.Ya., Kulakova, S.M., Isamailov, M.A. and Shkuropatova, V.A. (1986) Biochim. Biophys. Acta 849, 337–346.

[13] Visscher, K.J., Bergström, H., Sundström, V., Hunter, C.N. and Van Grondelle, R. (1989) Photosynth. Res., submitted.

[14] Rijgersberg, C.P., Van Grondelle, R. and Amez, J. (1980) Biochim. Biophys. Acta 592, 53–64.

[15] Förster, T.H. (1948) Ann. Phys. (Leipzig) 2, 55–75.

[16] Förster, T.H. (1949) Z. Naturforsch. A4, 321–327.

[17] Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597–609.

[18] Kirmaier, C., Holten, D. and Parson, W.W. (1985) Biochim. Biophys. Acta 810, 49–61.
[19] Sebban, P., Robert, B. and Jolchine, G. (1985) Photochem. Photobiol. 42, 573–578.
[20] Van Grondelle, R., Kramer, H.J.M. and Rijgersberg, P. (1982) Biochim. Biophys. Acta 682, 208–215.
[21] Moog, R.S., Kuki, A., Fayer, M.D. and Boxer, S.G. (1984) Biochemistry 23, 1564–1571.
[22] Bergström, H., Westerhuis, W.H.J., Sundström, V., Van Grondelle, R., Niedermann, R.A. and Gillbro, T. (1988) FEBS Lett. 233, 12–16.
[23] Shimada, K., Mimuro, M., Tamai, N. and Yamazaki, I. (1989) Biochim. Biophys. Acta, in press.
[24] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.
[25] Verméglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500–515.
[26] Yeates, T.O., Komiya, H., Rees, D.C., Allen, J.P. and Feher, G. (1987) Proc. Natl. Acad. Sci. USA 84, 6438–6442.