

# THE BALANCE BETWEEN PRIMARY FORWARD AND BACK REACTIONS IN BACTERIAL PHOTOSYNTHESIS

H. RADEMAKER AND A. J. HOFF, *Department of Biophysics, Huygens Laboratory of the State University, 2300 RA Leiden, The Netherlands*

**ABSTRACT** The temperature dependence of the bacteriochlorophyll fluorescence and reaction center triplet yield in whole cells of *Rhodospseudomonas sphaeroides* strain 2.4.1 and of the magnetic field-induced fluorescence increase are calculated, taking into account rate constants of losses in the antenna system and of charge separation and recombination in the reaction center. Triplet and singlet yield after recombination in the reaction center are described by the radical pair mechanism. Good fits of the theoretically calculated temperature dependence with published experimental results could be obtained, assuming that  $k_{\text{r}}$ , the rate constant for recombination of the charges on the primary donor  $P^+$  and the reduced intermediate acceptor  $I^-$  to the lowest excited singlet state  $P^*I$  of the reaction center bacteriochlorophyll, is temperature-dependent via the Boltzmann factor  $k_{\text{r}} \exp(-\Delta E/kT)$ , where  $\Delta E$  is the energy difference between  $P^*I$  and  $P^+I^-$  and  $k_{\text{r}}$  is the frequency factor.  $k_{\text{g}}$  and/or  $k_{\text{t}}$ , the rate constants for recombination to the singlet ground and triplet states, respectively, were assumed to be temperature-independent, or temperature-dependent via their exothermicity factors  $k_i = C_i T^{-1/2} \exp(-E_i/kT)$  with  $i = \text{g, t}$ . Depending on the particular choice for the temperature dependence of  $k_{\text{g}}$  and  $k_{\text{t}}$ , best fits were obtained for  $\Delta E = 45\text{--}75$  meV and recombination rate constants at 300 K of  $k_{\text{g}} = 0.4\text{--}0.8$  ns $^{-1}$ ,  $k_{\text{g}} = 0.08\text{--}0.12$  ns $^{-1}$ , and  $k_{\text{t}} = 0.3\text{--}0.5$  ns $^{-1}$ . The model predicts a lifetime of the radical pair  $P^+I^-$  that is somewhat larger than that of delayed fluorescence; a magnetic field increases both.

## INTRODUCTION

The photophysical processes in photosynthesis can be divided into essentially two classes: (a) the capture of a photon and the transfer of excitation energy to the so-called reaction center and (b) the separation of charges within this reaction center and their subsequent stabilization. The remarkably high efficiency of the latter processes must be understood in terms of a detailed balance between rates of excitation transfer and trapping, forward electron transport from the primary donor to the sequence of acceptors (comprising short-lived intermediates with a lifetime  $\tau$  of  $\leq 1$   $\mu\text{s}$  and secondary acceptors with  $\tau \geq 1$   $\mu\text{s}$ ), electron-hole recombination (to the ground state and/or the excited singlet and triplet states of the primary donor), and various other processes, such as excitation transfer back from the reaction center to the antenna system, radiative and radiationless decay of excited singlet states, and intersystem crossing from the excited singlet states to triplet states.

In this communication we will attempt to give a description of the photophysics of charge separation in bacterial photosynthesis in terms of the above mentioned reaction rates. We will focus on the kinetics and yield of electron-hole recombination processes, taking into account the spin statistics of radical pair recombination. Our input data will consist of a broad range of experimental results, drawn from fast (nano-, picosecond) optical spectroscopy, and fluores-

cence and electron-spin resonance data obtained on the bacterial photosystem in different states at temperatures between 80 and 300 K.

We will show that a consistent description can be given only when a relatively fast back reaction to the excited singlet state of the primary donor is taken into account. We determine an activation energy of 45–75 meV for this back reaction, depending on the assumed temperature-dependence of the recombination rates. The rate of this back reaction is shown to have only second-order influence on the overall quantum efficiency of charge separation.

## THEORETICAL

### *Introduction*

In this section we develop the theory for the calculation of the fluorescence and triplet yield. We first give the expressions for the fluorescence and triplet yields in terms of the probabilities of energy transfer to and from the trap, of energy losses by fluorescence, internal conversion and intersystem crossing, and of charge separation. Then the back reaction from the photoinduced pair  $P^+I^-$  (in which  $P$  is the primary donor, a bacteriochlorophyll dimer, and  $I$  the primary acceptor, bacteriopheophytin; see for a review of primary processes, e.g., reference 1) to the excited state  $P^*I$  is introduced, together with the decay of  $P^+I^-$  to the reaction center triplet state  $P^T I$  and to the ground state  $PI$ . This is done taking into account the time evolution of the spin statistics of the radical pair.

The resulting expressions for the fluorescence and triplet yield are used to calculate their temperature dependence in bacteria in which the forward electron transport is blocked by chemical reduction of the iron-ubiquinone complex  $X$ . It is assumed that the rate of the back reaction to  $P^*I$  is dependent on temperature via the Boltzmann factor  $k_{so} \exp(-\Delta E/kT)$ , where  $\Delta E$  is the energy difference between the  $P^*I$  and the  $P^+I^-$  states,  $T$  is the temperature, and  $k_{so}$  is the frequency factor.

In general, the recombination rates to  $PI$ ,  $k_g$  and to  $P^T I$ ,  $k_t$ , will also be temperature-dependent. As there are no tested models available for these dependences, we have taken several limiting cases, e.g., either  $k_g$  independent of temperature and  $k_t$  depending on  $T$  via its exothermicity factor (reference 2 and Results),  $k_t$  independent of  $T$  and  $k_g$  dependent via its exothermicity factor, or both  $k_g$  and  $k_t$  dependent on  $T$  via an exothermicity factor.

For each of the assumed modes of temperature-dependence of  $k_g$  and  $k_t$  the calculated temperature-dependence of both the emission and triplet yield is then fitted to the experimentally observed dependence in *Rhodopseudomonas sphaeroides* strain 2.4.1 (3).

Best fits are obtained for a nonzero value of the decay rate of  $P^+I^-$  to the ground state, but this rate is always appreciably slower than the rate of the back reaction to the excited state  $P^*I$ . The value of  $\Delta E$  resulting from the various fits lies in the range 45–75 meV, i.e., the energy level of the radical pair  $P^+I^-$  lies appreciably closer to that of  $P^*I$  than previously estimated (3, 4).

With the set of parameters resulting from the fitting procedure, the effect of a magnetic field on the yield of delayed fluorescence was also calculated. The results are compared with recently reported experiments (5). Furthermore, we give quantitative predictions for the temperature and magnetic field dependence of the lifetimes of the radical pair  $P^+I^-$  and of delayed fluorescence.

### Calculation of the Fluorescence and Triplet Yield in Whole Cells in State $P^+I^-X^-$

To calculate the temperature dependence of the fluorescence and triplet yield we assume that in whole cells the reaction centers (with fractional concentration  $R$ ) are embedded in a "lake" of bacteriochlorophyll (Bchl) molecules. The processes that take place in the antenna and reaction center are depicted in Fig. 1. Quanta, that are absorbed by Bchl are transferred to the reaction centers. During the transfer to the traps excitations are lost by fluorescence, intersystem crossing to the triplet state and internal conversion (rate constants  $k_f$ ,  $k_{isc}$ , and  $k_{ic}$ , respectively). The rate constant for loss in the antenna system is  $k_l = k_f + k_{isc} + k_{ic}$ . In the excited reaction centers an electron can be transferred from the primary donor  $P$  to the primary acceptor  $I$  with rate constant  $k_e$ .

Immediately after charge separation the spins of the radical pair  $P^+I^-$  are in a singlet ( $S = 0$ ,  $S$  is the total spin quantum number) configuration. Through the influence of magnetic interactions, which may differ for the two spins, this configuration is periodically converted into the triplet configuration ( $S = 1$ ; for details of this process see reference 6). From the singlet configuration  $(P^+I^-)^S$ , the charges may recombine to the ground state  $PI$  or to the excited singlet state  $P^*I$ ; from the triplet configuration  $(P^+I^-)^T$ , recombination yields the triplet state of the donor,  $P^T I$ . When the radical pair recombines to the singlet excited state  $P^*I$ , the excitation can be transferred back into the antenna system and the system is again in its initial state.

Fluorescence increases due to the decrease of the rate constant for energy transfer between antenna Bchl molecules become apparent only at temperatures below 60 K (7). Therefore we may assume that over the temperature range we consider (80–300 K), the energy transfer is fast with respect to all other processes (8, 9). As a consequence, the excitations are evenly distributed over all Bchl-molecules, including the reaction centers. The effective rate constant for trapping in the reaction centers is then  $k_e R$ .

The reaction center can have  $n$  different nuclear spin configurations denoted by  $i$ , each of

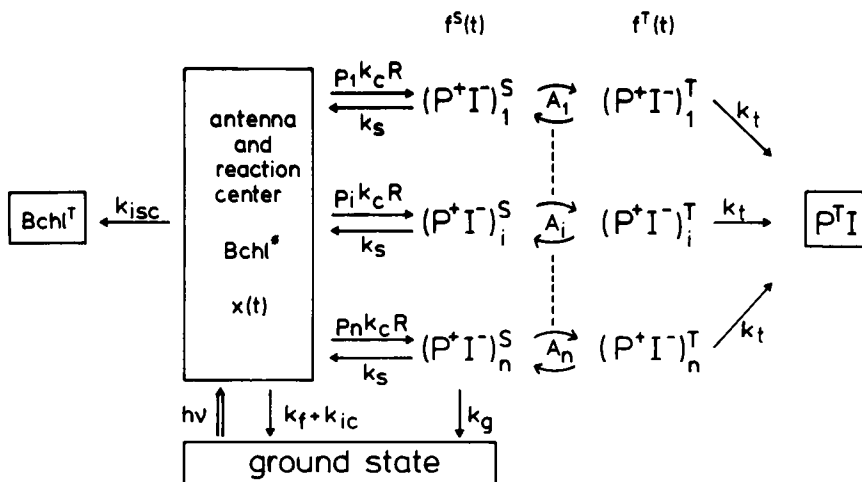


FIGURE 1 Scheme for energy transfer and electron redistribution reactions occurring in the antenna Bchl and the reaction center with reduced acceptor  $X$ . For an explanation of symbols see the List of Symbols.

which occurs with a probability  $p_i$ ,  $\sum_i p_i = 1$ . We assume that the reaction center remains in this spin state during the lifetime of the radical pair. The rate constant for formation of state  $(P^+I^-)_i$  from excited Bchl is  $p_i k_c R$ .

For a single turnover (i.e., when the radical pair recombines to  $P^*I$ , no recombination of charges is considered), the probability that a radical pair in nuclear spin state  $i$ , which is created at zero time, still exists at time  $t$  is denoted by  $y_i(t)$  [ $y_i(0) = 1$ ]. The probability that it exists in the singlet state at time  $t$  is given by  $y_i^S(t)$  [ $y_i^S(0) = 1$ ] and that for the triplet state by  $y_i^T(t)$  [ $y_i^T(0) = 0$ ];  $y_i^S(t) + y_i^T(t) = y_i(t)$ .

The single turnover probabilities for formation of the excited singlet state and the triplet state are given by

$$\phi_{si} = k_s \int_0^\infty y_i^S(t) dt = k_s Y_i^S(0) \quad (1)$$

and

$$\phi_{ti} = k_t \int_0^\infty y_i^T(t) dt = k_t Y_i^T(0), \quad (2)$$

in which  $Y_i^S(s)$  and  $Y_i^T(s)$  are the Laplace transforms of  $y_i^S(t)$  and  $y_i^T(t)$ , respectively.

We now proceed to calculate the kinetics of excited Bchl (fluorescence and luminescence kinetics) and of the radical pair, and the yields for photon emission and triplet state formation, using the scheme of Fig. 1. The kinetics of Bchl\*,  $x(t)$ , can be determined from the differential equation:

$$\frac{dx(t)}{dt} = -(k_1 + k_c R) x(t) + k_s \sum_{i=1}^n f_i^S(t), \quad (3)$$

in which  $f_i^S(t)$  is the concentration of  $(P^+I^-)_i^S$  at time  $t$ .  $f_i^S(t)$  can be expressed in  $x(t)$  and  $y_i^S(t)$  as follows. The number of charge separations from Bchl\* to the state  $(P^+I^-)_i^S$  between time  $u$  and  $u + du$  will be  $p_i k_c R x(u) du$ . The contribution of the pairs created in the time interval  $(u, u + du)$  to the kinetics of the subensemble of radical pairs  $(P^+I^-)_i^S$  is 0 for  $0 \leq t < u$  and  $p_i k_c R x(u) du y_i^S(t - u)$  for  $t > u$ .

Integration over all values of  $u < t$  yields  $f_i^S(t)$ , the concentration of  $(P^+I^-)_i^S$  at time  $t$ :

$$f_i^S(t) = p_i k_c R \int_0^t x(u) y_i^S(t - u) du + f_{i0}^S y_i^S(t), \quad (4)$$

in which  $f_{i0}^S$  is the concentration of  $(P^+I^-)_i^S$  at  $t = 0$ . Analogously, we have for  $f_i(t)$  and  $f_i^T(t)$ , the concentrations of  $(P^+I^-)_i$  and  $(P^+I^-)_i^T$ , respectively:

$$f_i(t) = p_i k_c R \int_0^t x(u) y_i(t - u) du + f_{i0} y_i(t) \quad (5)$$

and

$$f_i^T(t) = p_i k_c R \int_0^t x(u) y_i^T(t - u) du + f_{i0}^T y_i^T(t). \quad (6)$$

Substitution of Eq. 4 into Eq. 3 and Laplace transformation gives for  $X(s)$ , the Laplace transform of  $x(t)$ :

$$X(s) = \frac{x_0 + k_s \sum_i f_{i0}^S Y_i^S(s)}{s + k_1 + k_c R - k_s k_c R \sum_i p_i Y_i^S(s)}, \quad (7)$$

in which  $x_0$  is the concentration of Bchl\* at  $t = 0$ .

Laplace transformation of Eq. 5 and summation yields

$$F(s) = \sum_i F_i(s) = \sum_i [p_i k_c R X(s) Y_i(s) + f_{i0} Y_i(s)] \quad (8)$$

in which  $F_i(s)$  is the Laplace transform of  $f_i(t)$ , and  $F(s)$  that of  $f(t) = \sum_i f_i(t)$ , the total concentration of  $P^+I^-$ .

For the calculation of the fluorescence and triplet yields we take as initial conditions  $x_0 = 1$  and  $f_0 = 0$ . Eq. 7 then reduces to

$$X(s) = [s + k_1 + k_c R - k_s k_c R \sum_i p_i Y_i^S(s)]^{-1}. \quad (9)$$

The fluorescence yield is the total probability that Bchl\* will decay to the ground state under emission of a photon, and is given by

$$P_F = k_f \int_0^\infty x(t) dt = k_f X(0). \quad (10)$$

With Eqs. 1 and 9 this gives

$$P_F = \frac{k_f}{k_1 + k_c R(1 - \phi_s)}, \quad (11)$$

in which  $\phi_s = \sum_i p_i \phi_{si}$ , the average probability for  $P^+I^-$  to recombine to  $P^*I$ .

The reaction center triplet yield is the total probability that  $P^+I^-$  will decay to the triplet state and is given by

$$P_T^R = k_t \sum_i \int_0^\infty f_i^T(t) dt = k_t \sum_i F_i^T(0), \quad (12)$$

in which  $F_i^T(s)$  is the Laplace transform of  $f_i^T(t)$ .

Analogously to Eq. 8 we have (with initial condition  $f_{i0} = 0$ ):

$$F^T(s) = \sum_i F_i^T(s) = k_c R X(s) \sum_i p_i Y_i^T(s), \quad (13)$$

and the reaction center triplet yield is given by

$$P_T^R = \frac{k_c R \phi_t}{k_1 + k_c R(1 - \phi_s)}, \quad (14)$$

in which  $\phi_t = \sum_i p_i \phi_{ti}$ .

The total probability of triplet formation,  $P_T$ , also includes intersystem crossing, which is proportional to the fluorescence:  $P_T^{\text{isc}} = P_F k_{\text{isc}}/k_f$ , and is given by

$$P_T = \frac{k_{\text{isc}} + k_c R \phi_t}{k_1 + k_c R(1 - \phi_s)}. \quad (15)$$

To gain a physical understanding of Eqs. 11 and 15, it is useful to consider a simplified model, where all reaction centers are in the same nuclear spin state and, as a consequence, averaging over these spin states is not necessary. Let  $p_c$  be the single-turnover probability for charge separation when an excitation is present in the antenna system:  $p_c = k_c R / (k_1 + k_c R)$ , and  $p_f$  the probability that the excitation is lost as fluorescence:  $p_f = k_f / (k_1 + k_c R)$ . Since the charge separation is reversible (with probability  $\phi_s$ ), the process of charge separation and recombination goes on indefinitely, each time with combined probability  $p_c \phi_s$ . The total probability for fluorescence emission is then obtained by the sum:

$$P_F = p_f \sum_{n=0}^{\infty} (p_c \phi_s)^n = p_f / (1 - p_c \phi_s). \quad (16)$$

From this, Eq. 11 follows readily. The expression for the triplet yield (Eq. 15) can be obtained in a similar way.

### *The Decay Time of $P^+I^-$ and of Delayed Light Emission*

For a single turnover we define the lifetime of the radical pair by

$$\tau'_i = \int_0^{\infty} y_i(t) dt = Y_i(0) \quad (17)$$

in which  $Y_i(s)$  is the Laplace transform of  $y_i(t)$ . The lifetime thus defined is identical to the usual one for an exponential decay.

Since  $y_i(t) = y_i^S(t) + y_i^T(t)$ , it follows from the definitions of  $\tau'_i$ ,  $\phi_{si}$ , and  $\phi_{ti}$  that

$$\tau'_i = \frac{\phi_{si}}{k_s} + \frac{\phi_{ti}}{k_t}. \quad (18)$$

We define the decay time  $\tau_{rp}$  of  $P^+I^-$  as

$$\tau_{rp} = \frac{1}{f_0} \int_0^{\infty} f(t) dt = F(0)/f_0, \quad (19)$$

and that of delayed light emission  $\tau_{dl}$  as

$$\tau_{dl} = \frac{1}{x_0} \int_0^{\infty} x(t) dt = X(0)/x_0. \quad (20)$$

To separate the rise kinetics of  $P^+I^-$  from its decay kinetics and prompt fluorescence from delayed fluorescence, we take as initial conditions equilibrium concentrations of Bchl\* and  $(P^+I^-)_i^S$  ( $i = 1, n$ ):  $k_s f_{i0}^S = p_i k_c R x_0$ . Summation yields  $k_s f_0^S = k_c R x_0$  with  $f_0^S = \sum_i f_{i0}^S$ , hence  $x_0 = f_0^S k_s / k_c R$  and  $f_{i0}^S = p_i f_0^S$ . Since the dephasing of the spins is much slower than the rate of charge separation, the equilibrium will be reached before an appreciable triplet population has been formed. Therefore we may assume that at zero time all radical pairs are in the singlet state, thus  $f_{i0}^S = f_{i0}$  and  $f_0^S = f_0$ . Substitution of  $x_0$ ,  $f_{i0}$ , and  $f_0^S$  into Eqs. 7 and 8 yields

$$F(s) = (k_c R X(s) + f_0) \sum_i p_i Y_i(s) \quad (21)$$

and

$$X(s) = f_0 \frac{k_s/k_c R + k_s \sum_i p_i Y_i^S(s)}{s + k_1 + k_c R - k_s k_c R \sum_i p_i Y_i^S(s)} \quad (22)$$

Substitution of these expressions into Eqs. 19 and 20 yields, with help of Eqs. 1 and 17 for the lifetime of  $P^+I^-$ :

$$\tau_{rp} = \frac{k_1 + k_c R + k_s}{k_1 + k_c R(1 - \phi_s)} \tau', \quad (23)$$

and for the lifetime of the delayed fluorescence:

$$\tau_{dl} = \frac{1 + k_c R(\phi_s/k_s)}{k_1 + k_c R(1 - \phi_s)}, \quad (24)$$

in which  $\tau'$  is the lifetime of  $P^+I^-$  for a single turnover (Eq. 17), averaged over all nuclear spin configurations:  $\tau' = \sum_i p_i \tau_i'$ . When  $\phi_s$  and  $\phi_i$  are known,  $\tau'$  can be calculated from Eq. 18.

Eqs. 23 and 24 cannot easily be derived in an intuitive way. One would expect that the decay times of radical pair and excited Bchl are lengthened by the reversible process of charge separation and recombination in the same manner as the fluorescence yield, viz. by the factor  $(1 - p_s \phi_s)^{-1}$  (Eq. 16), but the exact form of the numerator in Eqs. 23 and 24 depends on the choice of initial conditions.

It is seen that in general the decay times of  $P^+I^-$  and of delayed light emission are not equal.

#### *Calculation of the Single Turnover Probabilities for Singlet and Triplet Formation, $\phi_s$ and $\phi_i$*

For calculation of  $\phi_i$ , the average probability of recombination of the radical pair to the triplet state  $P^T$ , we use the formulas of Haberkorn and Michel-Beyerle (10), that are based on the so-called radical pair mechanism. In this model the interconversion of the singlet and triplet configuration of the radical pair is governed by the difference in local magnetic field experienced by each of the two radicals. For  $P^+$  and  $I^-$  the spin dephasing is determined by the hyperfine field, both in zero (earth) and high (a few tenths of a Tesla) magnetic fields. In the treatment of Haberkorn and Michel-Beyerle the many different hyperfine fields are concentrated to one hyperfine field of one fictitious nuclear spin, with hyperfine interaction  $A$ . The yield of formation of  $(P^+I^-)^T$ , and consequently the yield of  $P^TI$ , depends on the number of triplet sublevels of the radical pair that are close enough to its singlet level to give an appreciable interconversion coefficient. In zero magnetic field the triplet sublevels will all be close to the singlet level, although the precise amount of interconversion for each of them depends on the strength of dipolar and exchange interactions between the unpaired spins of the two radicals. In a high magnetic field  $H$  only the  $m_s = 0$  (where  $m_s$  is the magnetic quantum number) triplet sublevel is close enough to the singlet level for singlet-triplet mixing to occur, the  $m_s = \pm 1$  levels being separated from the triplet level by the Zeeman energy  $\gamma H$ , where  $\gamma$  is the gyromagnetic ratio.

In zero magnetic field, starting with all  $P^+I^-$  in the singlet configuration  $(P^+I^-)^S$ ,  $\phi_i(0)$ , the single turnover probability of recombination to  $P^+I$  is, in our notation, given by

$$\phi_i(0) = \frac{3A^2k_i(k_s + k_g + k_t)}{[3A^2 + 4k_t(k_s + k_g)](k_s + k_g + k_t)^2 + 16k_t(k_s + k_g)(2J - A/2)^2}. \quad (25)$$

In high magnetic field (neglecting dephasing of the spins by difference in  $g$ -values of  $P^+$  and  $I^-$ ) this probability is given by

$$\phi_i(\infty) = \frac{A^2k_i(k_s + k_g + k_t)}{[A^2 + 4k_t(k_s + k_g)](k_s + k_g + k_t)^2 + 16k_t(k_s + k_g)(2J)^2}. \quad (26)$$

$J$  is the exchange integral; the rate constants  $k_s$ ,  $k_g$ , and  $k_t$  are defined in Fig. 1.

The value of  $\phi_s$  is given by

$$\phi_s = \frac{k_s}{k_s + k_g} (1 - \phi_i). \quad (27)$$

### *The Quantum Efficiency of Charge Separation*

The quantum efficiency of stable charge separation (i.e.,  $P^+IX^-$  formation) starting from  $PIX$ , can be calculated in a similar way as the reaction center triplet yield in the state  $PIX^-$ . The total probability of  $X^-$  formation,  $P_x$ , is given by

$$P_x = \frac{k_c R \phi_x}{k_i + k_c R (1 - \phi_{so})}, \quad (28)$$

in which  $\phi_x$  is the single turnover probability for  $X^-$  formation from  $P^+I^-X$  and  $\phi_{so}$  the probability of recombination to  $P^+IX$ . It can be easily shown that the yield of reaction center triplet formation from  $P^+I^-X$  is negligible. Hence,  $\phi_x \approx k_r/(k_s + k_g + k_r)$  and  $\phi_{so} \approx k_s/(k_s + k_g + k_r)$ , in which  $k_r$  is the rate constant for reduction of  $X$  by  $I^-$ . It follows that

$$P_x = \left[ 1 + \frac{k_i}{k_c R} + \frac{k_g}{k_r} + \frac{k_i}{k_c R} \left( \frac{k_g}{k_r} + \frac{k_s}{k_r} \right) \right]^{-1}. \quad (29)$$

From Eq. 29 it is seen that the contribution to the loss due to the back reaction to  $P^*$  is of second order compared to the losses due to the recombination to the ground state  $P$  and the losses occurring in the antenna system. Hence, the notion that a back reaction to  $P^*$  is detrimental to photosynthetic quantum efficiency should be qualified. As long as  $k_s/k_r < 1$ , loss due to the back reaction to  $P^*$  is negligible or comparable to other loss processes.

### *The Relation between Fluorescence and Triplet Yields*

Since  $(1 + k_g/k_s)\phi_s + \phi_i = 1$  (Eq. 27), we can eliminate  $\phi_s$  and  $\phi_i$  from the equations for the fluorescence yield (Eq. 11) and the triplet yield (Eq. 15) and arrive at the relation between  $P_F$  and  $P_T$ :

$$P_T + bP_F = a, \quad (30)$$

in which

$$a = 1 + k_g/k_s \quad (31)$$



and

$$b = (ak_i - k_{isc} + k_c R k_g / k_s) / k_t. \quad (32)$$

This relationship is valid, independent of the mechanism of reaction center triplet formation. We assume that the magnetic fields employed do not change the rate constants and only affect the values of  $\phi_s$  and  $\phi_t$ .

For the ratio  $Z$  of the relative variations of triplet and fluorescence yields, one can then derive from Eqs. 30–32,

$$Z = - \left( \frac{\Delta P_T}{P_T} \right) / \left( \frac{\Delta P_F}{P_F} \right) = \frac{a}{P_T} - 1, \quad (33)$$

in which  $P_T$  is the triplet yield in zero magnetic field and  $\Delta P_T = (P_T)_B - (P_T)_{B=0}$  the change of this yield caused by a magnetic field.  $P_F$  and  $\Delta P_F$  are defined analogously. It is seen that, at a fixed temperature, the relative changes of emission and triplet yield are linearly related. Hence  $\Delta P_F / P_F$  and  $\Delta P_T / P_T$  should saturate and reach their half-saturation values ( $B_{1/2}$ ) at the same magnetic field strength. For *Rps. sphaeroides* this is indeed observed (5, 11).

## RESULTS

### *Fitting the Theoretical to the Experimental Values*

For the calculation of the total emission and triplet yield the following procedure is adopted: We assume that in the reaction center  $k_s$  is temperature dependent with activation energy  $\Delta E$ :

$$k_s = k_{s0} \exp(-\Delta E / kT) \quad (34)$$

For the temperature dependence of  $k_g$  and  $k_t$ , four limiting cases were considered: (a)  $k_g$  and  $k_t$  depend on temperature via their exothermicity factors:

$$k_g = C_g T^{-1/2} \exp(-E_g / kT) \quad (35)$$

and

$$k_t = C_t T^{-1/2} \exp(-E_t / kT); \quad (36)$$

(b)  $k_g$  is independent of temperature and  $k_t$  depends on the temperature via Eq. 36; (c)  $k_t$  is independent of temperature and  $k_g$  depends on temperature via Eq. 35; (d) both  $k_g$  and  $k_t$  are independent of temperature.

Eqs. 35 and 36 follow from the high temperature limit of the equations for the rate of electron transfer by vibronically coupled tunneling as derived by Hopfield (2). The activation energies  $E_i$  ( $i = g, t$ ) are given by  $E_i = (\delta E_i - \Delta_i)^2 / 4\Delta_i$ , where  $\Delta_i$  is the Franck-Condon parameter,  $\delta E_i$  is the energy difference between initial and final states;  $C_i$  is a frequency factor.

The effective rate constant for charge separation,  $k_c R$ , is estimated from the fluorescence lifetime, measured in *Rps. sphaeroides* 2.4.1 in state  $P^+ I^- X^-$ . In this state the lifetime is approximately given by  $\tau_f \approx (k_i + k_c R)^{-1} \approx 100$  ps (12). Since  $k_i \ll k_c R$ , it follows that  $k_c R \approx 10$  ns<sup>-1</sup>. This value is in good agreement with the observed rate constant for charge separation

in reaction center preparations ( $k_c \approx 0.3 \text{ ps}^{-1}$  [13], if we take for  $R$  the ratio of the concentration of reaction center Bchl to that of Bchl-protein complexes ( $R \approx 1/30$ , [14]). Assuming a loss of 10%, it follows that  $k_1 \approx 1 \text{ ns}^{-1}$ . This results in a fluorescence lifetime of 1 ns when no trapping occurs, in good agreement with the measured lifetime in the reaction centerless mutant PM 8 dpl of *Rps. sphaeroides* (12). The loss of 10% is also in agreement with the fluorescence increase to  $\sim 10$  times the  $P_{F0}$ -level (7) upon lowering the temperature to 4.2 K. For the rate constant for fluorescence,  $k_f$ , the value calculated from the absorption spectrum  $k_f \approx 0.06 \text{ ns}^{-1}$  (15) is used. The rate constant for intersystem crossing,  $k_{isc}$ , is estimated to be  $\sim 30\%$  higher for Bchl in vitro than that for fluorescence (16, 17), hence  $k_{isc} \approx 0.08 \text{ ns}^{-1}$ . The temperature dependence of  $k_c$  was found to be very small (18) and is neglected. The rate constants  $k_1$ ,  $k_{isc}$ , and  $k_f$  are assumed to be independent of temperature. From the linewidth of the  $P^+$  ESR-signal, the hyperfine splitting parameter can be estimated:  $A \approx 0.23 \text{ ns}^{-1}$  (10, 19). The exchange parameter  $|J|$  was found to lie in the range 0.1–0.5 mT (20), equivalent to  $|J| = 0.02\text{--}0.1 \text{ ns}^{-1}$ . The fitting procedure was followed for values of  $J$ , ranging from  $-0.2$  to  $+0.2 \text{ ns}^{-1}$  in steps of  $0.05 \text{ ns}^{-1}$ .

From the adjustable parameters  $k_{so}$ ,  $\Delta E$ ,  $k_g$  (or  $C_g$  and  $E_g$ ) and  $k_i$  (or  $C_i$  and  $E_i$ ) we calculate  $\phi_s$  and  $\phi_t$  in zero magnetic field, using Eqs. 34–36, 25, and 27. With the help of Eqs. 11 and 15 the fluorescence and triplet yield are calculated. The triplet yields measured by Van Grondelle et al. (3), are carotenoid-triplet yields. However, since the transfer of the triplet excitation from Bchl to carotenoid occurs very rapidly in the temperature range we study, the carotenoid triplet yield is equal to the Bchl-triplet yield (21). Since the experimental values of the emission and triplet yield (3) are relative values, we normalized the experimental values for  $P_F$  by making their sum equal to the sum of the theoretically calculated points, thus making the sum of the deviations between theoretical and experimental curves zero. The experimental triplet yields were normalized to a yield of 0.16 at 294 K (22). The value of the “goodness of the fit”  $G$  is obtained by adding the squares of the deviations between theoretical and normalized experimental values. To ensure that the deviations of emission and triplet yield had about equal weight factors, the deviations for  $P_F$  and  $P_T$  were each normalized to the respective yields, averaged over the whole temperature region.  $\Delta E$ ,  $k_{so}$ ,  $C_g$  and  $E_g$  (or  $k_g$ ),  $C_i$  and  $E_i$  (or  $k_i$ ) were varied until a minimum value of  $G$  was reached. In this way a least-squares fit is obtained to the experimental data for the temperature dependence of the fluorescence and triplet yield from reference 3 simultaneously.

It should be noted that a good fit for the theoretically calculated values to the experimental data for either the fluorescence yield or the triplet yield alone can be obtained for various sets of the six adjustable parameters. However, the values of these parameters to obtain a good fit to the triplet and fluorescence data simultaneously lie in a narrow range.

### Best Fit Results

The result of the fitting procedure described in the previous section is displayed in Fig. 2 for two types of temperature dependence for  $k_g$  and  $k_i$ . For case *a* (variable  $k_g$  and  $k_i$ ) the best fit (solid line) is obtained for  $J = -0.05 \text{ ns}^{-1}$  (see below),  $k_{so} = 7.5 \text{ ns}^{-1}$ ,  $\Delta E = 72 \text{ meV}$ ,  $C_g = 7.4 \text{ ns}^{-1} \text{ K}^{1/2}$ ,  $E_g = 33.5 \text{ meV}$ ,  $C_i = 29 \text{ ns}^{-1} \text{ K}^{1/2}$ , and  $E_i = 31.4 \text{ meV}$ . The standard deviation of the fit is  $6 \times 10^{-4}$  for the fluorescence and  $3.0 \times 10^{-2}$  for the triplet yield. At room temperature the recombination rate constants are  $k_s = 0.43 \text{ ns}^{-1}$ ,  $k_g = 0.12 \text{ ns}^{-1}$ , and  $k_i = 0.49 \text{ ns}^{-1}$ . The

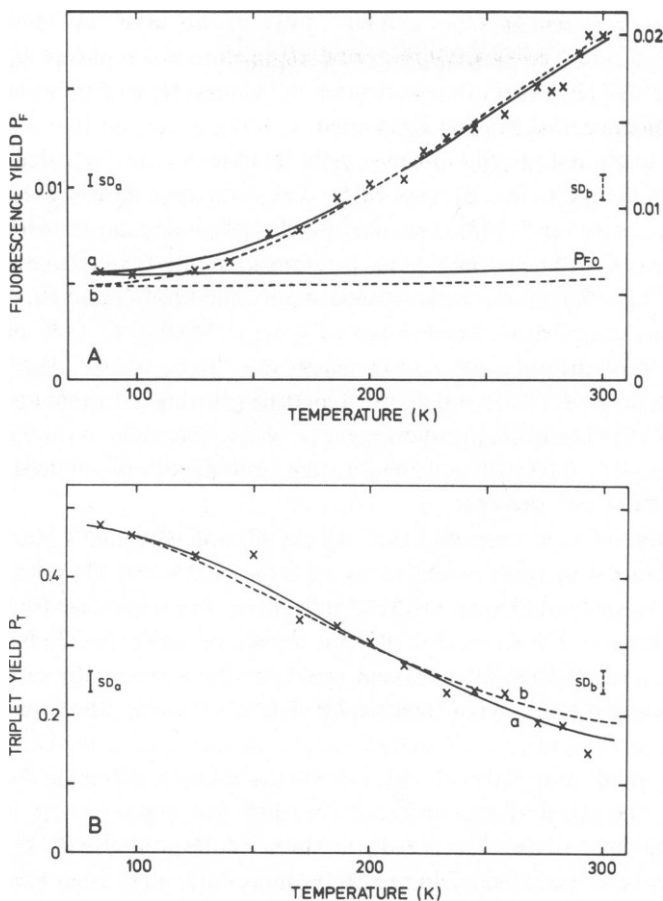


FIGURE 2 (A) Total (prompt plus delayed) fluorescence yield of *Rps. sphaeroides* whole cells as a function of temperature. (B) Triplet yield as a function of temperature. x, experimental points from reference 3; the lines represent the theoretically calculated best-fit dependence for case a (solid lines, left scale) and case b (broken lines, right scale) in state  $P I X^-$  and  $P I X$  (fluorescence yield only:  $P_{F0}$ ). Only average experimental values are shown. The indicated standard deviations (SD) are calculated taking into account all experimental points of reference 3. The following parameters were used to obtain the curves. Case a:  $k_{s0} = 7.5 \text{ ns}^{-1}$ ,  $\Delta E = 72 \text{ meV}$ ,  $C_g = 7.4 \text{ ns}^{-1} \text{ K}^{1/2}$ ,  $E_g = 33 \text{ meV}$ ,  $C_i = 29 \text{ ns}^{-1} \text{ K}^{1/2}$ ,  $E_i = 31 \text{ meV}$ ,  $J = -0.05 \text{ ns}^{-1}$ . Case b:  $k_{s0} = 4.4 \text{ ns}^{-1}$ ,  $\Delta E = 44 \text{ meV}$ ,  $k_g = 0.08 \text{ ns}^{-1}$ ,  $C_i = 6.7 \text{ ns}^{-1} \text{ K}^{1/2}$ ,  $E_i = 8.4 \text{ meV}$ ,  $J = 0.05 \text{ ns}^{-1}$ . For both cases,  $k_c R = 10 \text{ ns}^{-1}$ ,  $k_i = 1 \text{ ns}^{-1}$ ,  $k_f = 0.06 \text{ ns}^{-1}$ ,  $k_{isc} = 0.08 \text{ ns}^{-1}$ ,  $A = 0.23 \text{ ns}^{-1}$ .

best fit for case b (fixed  $k_g$ , variable  $k_i$ ; broken line) is obtained for  $J = 0.05 \text{ ns}^{-1}$ ,  $k_{s0} = 4.4 \text{ ns}^{-1}$ ,  $\Delta E = 44 \text{ meV}$ ,  $k_g = 0.08 \text{ ns}^{-1}$ ,  $C_i = 6.7 \text{ ns}^{-1} \text{ K}^{1/2}$ ,  $E_i = 8.4 \text{ meV}$ , with standard deviations of  $1.5 \times 10^{-3}$  for the fluorescence and  $3.2 \times 10^{-2}$  for the triplet yield. The resulting values for the rate constants at room temperature are:  $k_s = 0.77 \text{ ns}^{-1}$ ,  $k_g = 0.08 \text{ ns}^{-1}$ , and  $k_i = 0.28 \text{ ns}^{-1}$ . When  $k_g$  is varied with temperature via Eq. 35 and  $k_i$  is kept constant (case c) a similar fit for  $P_F$  and  $P_T$  is obtained. However, if both  $k_g$  and  $k_i$  are kept constant (case d), the fitting procedure did not converge, and no fit to the experimental data could be obtained.

The best fit parameters are not very sensitive to the normalization of the experimental

values of  $P_T$ . When the triplet yield at room temperature is taken to be 0.3 instead of 0.16 (i.e., a triplet yield at 80 K close to 1), the parameters change by <20%, except  $E_g$ , which becomes 60 instead of 33 meV. Hence, the rate constants lie within the range that is found when the temperature dependence of  $k_g$  and/or  $k_i$  is varied.

In Fig. 2 we also plotted the fluorescence yield in state  $P^+ I X$ ,  $P_{Fo}$ , calculated from Eq. 1 with  $\phi_s = \phi_{so}$  (compare Eq. 28).  $\phi_{so}$  was calculated with the rate constant for the reaction  $P^+ I^- X \rightarrow P^+ I X^-$ ,  $k_r \approx 5 \text{ ns}^{-1}$  (13). The increase of ~10% when the temperature is increased from 80 to 300 K is in good agreement with the experimentally found increase (3).

In case *a* equally good fits, i.e., similar standard deviations for  $P_F$  and  $P_T$ , could be obtained for values of the exchange parameter  $J$  ranging from  $-0.2$  to  $0.2 \text{ ns}^{-1}$ . Of the six parameters, only  $C_i$  is sensitive to the value of  $J$ . Increasing  $|J - A/4|$  for  $A = 0.23 \text{ ns}^{-1}$  from 0.01 ( $J = 0.05 \text{ ns}^{-1}$ ) to 0.11  $\text{ns}^{-1}$  ( $J = -0.05 \text{ ns}^{-1}$ ) increases the best fit value for  $C_i$  from  $6.6 \text{ ns}^{-1} \text{ K}^{1/2}$  to  $29 \text{ ns}^{-1} \text{ K}^{1/2}$ . The other parameters vary <15%. The drastic increase of the triplet decay rate is necessary to compensate for the lower probability of singlet-triplet conversion caused by the exchange interaction.

Because the value of  $C_i$  cannot be measured directly, it is not possible to determine the value of  $J$  from the best fit value of this rate parameter. However, the temperature at which the maximum in the calculated magnetic field-induced emission increase (see below) occurs is sensitive to the value of  $J$ . For  $J = -0.05 \text{ ns}^{-1}$  the maximum occurs at 235 K. For other values of  $J$  in the range  $-0.2$ – $0.2 \text{ ns}^{-1}$  the maximum is found at lower temperatures. As we have experimentally obtained a maximum close to 250 K (5), we choose the value  $J = -0.05 \text{ ns}^{-1}$  for the fit.

For case *b*, the standard deviation for  $P_T$  is not affected by a change in  $J$  from  $-0.2$  to  $0.2 \text{ ns}^{-1}$ . In contrast, the standard deviation for  $P_F$  showed a minimum for  $J = +0.05 \text{ ns}^{-1}$ . Changing this value by  $\pm 0.1 \text{ ns}^{-1}$  increased the standard deviation for  $P_F$  by a factor of 2. In this case the decrease of the triplet yield caused by an increase of  $|J - A/4|$  is compensated by a decrease of the best fit-value of  $k_g$ . For the results displayed in Fig. 2 the optimum value ( $J = 0.05 \text{ ns}^{-1}$ ) was used.

When all protons in the radical pair are replaced by deuterons,  $A$  decreases to  $0.12 \text{ ns}^{-1}$ . Keeping the rate constants fixed, we calculate for case *a* for this value of  $A$  a triplet yield at 300 K of  $P_T = 0.07$ , which is to be compared with the calculated triplet yield in normal (protonated) cells of 0.17. This is in serious disagreement with experiments on deuterated cells and reaction centers of *Rhodospirillum rubrum* (17, 23), where no effect of deuteration on the triplet yield was found. In contrast, the calculated magnetic field-induced decrease of the triplet yield is about equal, for  $A = 0.23 \text{ ns}^{-1}$  ( $\Delta P_T/P_T = -0.48$ ) and for  $A = 0.12 \text{ ns}^{-1}$  ( $\Delta P_T/P_T = -0.41$ ). This agrees with the experimental finding (17) that deuteration does not affect the magnetic field effects on the triplet yield. The effect of a decrease of  $A$  on the triplet yield and on the magnetic field-induced decrease of this yield is similar for cases *b* and *c*.

### *The Temperature Dependence of the Magnetic Field Effects on Total Fluorescence Emission and on the Triplet Yield*

With help of Eq. 26 the emission and triplet yields in high magnetic field were calculated for the best-fit parameters for the rate constants.

In Fig. 3, the calculated temperature dependence of the magnetic field-induced relative

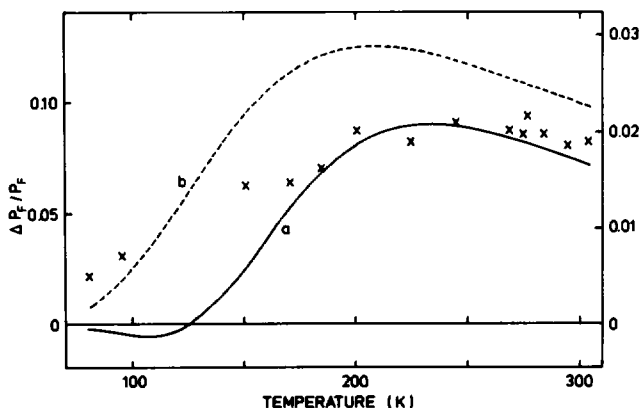


FIGURE 3 Magnetic field-induced relative change of total Bchl-emission as a function of temperature.  $\times$ , experimental points from reference 5 (right scale). Drawn line and broken line: theoretically calculated curves for case *a* and *b*, respectively (left scale).

increase in total Bchl-emission ( $\Delta P_F/P_F$ ) is displayed, together with the experimental results from reference 5. The theoretical values for case *a* ( $k_g$  and  $k_t$  variable; solid line) are arbitrarily normalized to the observed values at 250 K. The broken line represents the case when  $k_g$  is independent of temperature (case *b*). It is seen that for both types of temperature dependence a maximum in the  $\Delta P_F/P_F$  curve is predicted. Voznyak et al. (24) also observed a maximum in the  $\Delta P_F/P_F$  vs. temperature curve in whole cells and reaction center preparations of *Rps. sphaeroides* R 26. However, quantitatively the model predicts too large a magnetic field effect both for the emission (5) and for the triplet yield (calculation not shown) (11) in whole cells.

#### *The Temperature Dependence of the Decay Times of the Radical Pair and of Delayed Fluorescence*

Substituting the best fit rate constants that were used for the fit of Fig. 2, into Eqs. 23 and 24, we have calculated the lifetimes of the radical pair,  $\tau_{rp}$ , and of delayed fluorescence emission,  $\tau_{dl}$ , both in zero and in infinite magnetic field over the temperature region 80–300 K. The result of this calculation is shown in Fig. 4. The solid lines represent case *a* where all recombination rate constants are dependent on temperature according to Eqs. 34–36; the broken lines represent case *b* with  $k_g$  independent of temperature. At room temperature both cases predict a lifetime of delayed luminescence of 5–5.5 ns (curves 1) and a lifetime of the radical pair that is  $\sim 20\%$  longer (5.9–6.6 ns [curves 3]). For case *a* the predicted decay times increase sharply at temperatures below  $\sim 200$  K, due to the exponential temperature dependence of the rate constants. When  $k_g$  (or  $k_t$ , data not shown) is kept constant, the increase of the lifetimes is much less pronounced. Van Bochove et al. (25) measured delayed fluorescence of chromatophores of *Rps. sphaeroides* G1C under reducing conditions. The delayed fluorescence showed a nearly exponential decay with, at room temperature, a decay time of 8 ns and an amplitude about equal to that of prompt fluorescence. Upon lowering the temperature, the amplitude of the delayed light emission decreased monotonically and the

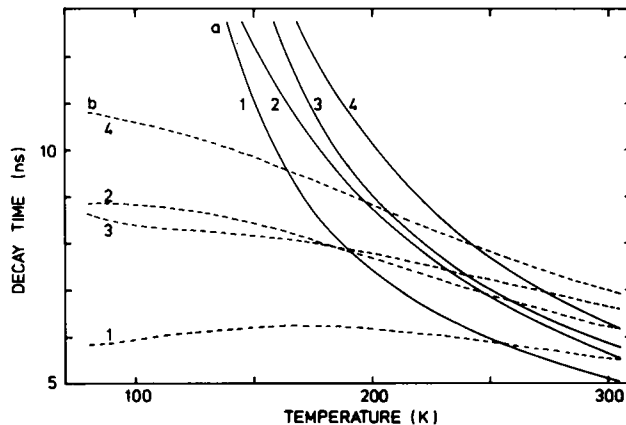


FIGURE 4 Calculated temperature dependence for the decay time of delayed Bchl-emission in zero (curves 1) and high (2) magnetic field and of the decay time of the radical pair  $P^+I^-$  in zero (3) and high (4) magnetic field. The solid lines represent case *a* and the broken lines case *b*.

lifetime remained constant down to  $\sim 200$  K. Below this temperature the lifetime could not be measured accurately, due to the low amplitude of the delayed fluorescence.

The broken lines of Fig. 4 (case *b*) are in much better agreement with the experiments than the solid lines, indicating that the temperature dependence of at least one of the rate constants is much less pronounced than assumed in Eqs. 35 and 36.

As borne out by Fig. 4, over the temperature range investigated a magnetic field increases the lifetime of both radical pair and delayed fluorescence, due to the decrease of the probability of triplet formation. Such an increase is indeed observed in bacterial reaction centers (W. W. Parson, personal communication) and also in spinach chloroplasts (26).

## DISCUSSION

The main aim of this paper is to describe the charge separation process within a consistent framework of reaction rates of energy transfer, energy loss, and electron transport, both backward and forward. It is shown that all reactions are tied together, and that it is not possible to derive data on separate reactions, such as activation energies, without taking the whole complex of reactions into consideration. A specific example is the activation energy  $\Delta E$  of the back reaction  $P^+I^- \rightarrow P^*I$ , which we find to lie in the range 45–75 meV by fitting equations giving the yield of fluorescence and reaction center triplets in terms of the above mentioned reaction rates to experimental results. To a first approximation the temperature dependence of the energetically uphill reaction  $P^+I^- \rightarrow P^*I$  is described by the product of the Boltzmann factor and the exothermicity factor for forward charge separation. This last factor is found to be very small (18). Consequently, we believe that the activation energy is solely due to the Boltzmann factor, so that the energy of 45–75 meV we find reflects the energy difference between the vibrational ground state levels of the excited donor and the  $P^+I^-$ -complex. The lower value, which is found when only one of the rate constants  $k_g$  and  $k_i$  is temperature dependent, gives a much better fit of the calculated to the experimental temperature dependence of the lifetime of delayed fluorescence (Fig. 4 and reference 25) than

the higher values of  $\Delta E$ . Therefore we expect the energy difference between  $P^*I$  and  $P^+I^-$  to be close to 45 meV.

Our values for the activation energy are in good agreement with the value found by Borisov ( $0.05 \pm 0.03$  eV) (27), but are appreciably lower than that reported by van Grondelle et al. (130 meV) (3) and by Shuvalov and Klimov (120 meV) (4). Van Grondelle et al. (3) calculated the activation energy  $\Delta E$  from the fluorescence data in the high temperature region employing a simple kinetic model, and using for the maximal fluorescence yield the experimental fluorescence yield in bacteria in the state  $P^+IX^{(-)}$ . This procedure does not take into account quenching by  $P^+$ . In our fitting procedure, we have used the experimentally determined lifetime of the fluorescence of a reaction centerless mutant (Results and reference 12). Moreover, in our model the time evolution of the radical pair and decay to the singlet ground state is explicitly taken into account. Shuvalov and Klimov (4) calculated  $\Delta E$  from an "Arrhenius" plot of the amplitude of a 10-ns luminescence component. Since the amplitude of the luminescence depends on  $k_s$  in a rather complicated way, the slope of their Arrhenius plot is not directly related to  $\Delta E$ .

The energy of the lowest excited singlet state  $P^*I$  is  $\sim 1.4$  eV above the ground state (28). The redox potential of the primary donor is  $E_m(P/P^+) = 0.45$  eV and that of the intermediary acceptor bacteriopheophytin  $E_m(I/I^-) = -0.55$  eV (29). On the basis of these redox potentials, a free energy difference between  $P^*$  and  $P^+I^-$  of  $\sim 0.4$  eV would be expected. The energy difference  $\Delta E$  we find is much lower. This is perhaps not surprising. From solution photochemistry studies it is well known that the energy level of an exciplex (a closely coupled radical pair) lies in general much higher than that of the same radical pair when it has diffused apart. The energy level of the exciplex with respect to that of the excited donor-acceptor complex depends strongly on the polarity of the solvent because of screening effects (reference 30 and H. Staerk, personal communication) and on the coordination of the central magnesium atom (31). As the bacterial donor-acceptor complex is buried inside a protein containing many polar groups, the position of the level of the exciplex  $P^+I^-$  may be tuned such that it is close enough to the  $P^*I$  level that forward electron transport is not impeded by the exothermicity factor of the reaction  $P^*I \rightarrow P^+I^-$ , but not so close that the back reaction  $P^+I^-$  lowers the quantum efficiency of charge separation too much.

Our fitting procedure contains assumptions on the temperature dependence of all back reaction rates. As discussed above, the assumption that  $k_s$  is temperature activated by a Boltzmann factor is probably realistic. For  $k_g$  and  $k_t$  the situation is much less clear. The reaction to the singlet ground state of  $P$  and its triplet state are both exothermic, and it seems reasonable to describe the temperature dependence of their rates by the well-known exothermicity factor (Eq. 35). However, up to now there is very little evidence that the rate of strongly exothermic reactions is indeed strongly (exponentially) dependent on temperature (see, e.g., reference 32). We have therefore not only fitted with an exothermicity factor for  $k_g$  and  $k_t$  but also for either  $k_g$  or  $k_t$ , or both, independent of temperature. For  $k_g$  and  $k_t$  temperature independent, we could not fit the data on the temperature dependence of the fluorescence. For the other combinations reasonable fits were obtained (Fig. 2), albeit that the fit with a thermal dependence for both  $k_g$  and  $k_t$  was somewhat better. The temperature dependence of the lifetimes of delayed fluorescence and of the radical pair, however, is much more sensitive to which combination is chosen. As seen from Fig. 4, the predicted lifetime of

the delayed fluorescence rises sharply with decreasing temperature when both  $k_g$  and  $k_i$  are exponentially dependent on temperature, whereas for either  $k_g$  and  $k_i$  constant, the lifetime does not increase appreciably over the range 300–200 K. Recent experiments by van Bochove et al. (25) are consistent with the latter result. Hence, we conclude that one of the rates  $k_g$  or  $k_i$  is little dependent on temperature. We cannot, at present, decide which one.

The functional form of the exothermic temperature dependence is only one of many conceivable forms; it has been chosen for its simplicity and because it has at least some theoretical foundation. We stress, however, that the activation energy and frequency factors for  $k_g$  and for  $k_i$  that result from the fits should be regarded as mathematical parameters describing an “experimental” temperature dependence as they may not have physical meaning.

Our value for the frequency factor ( $k_{so}$ ) of the back reaction to the excited singlet state is much lower than the rate of charge separation  $k_c$ . If electron transfer takes place via vibronically coupled tunneling, then the frequency factor is proportional to the square of the tunneling matrix element (the wave function overlap integral) which for the forward and back reaction is the same when only one electron donating and electron accepting orbital is considered (2). Thus, one would expect  $k_{so} \approx k_c$ . The rather large deviation we find might be due to rearrangement of the donor-acceptor geometry as a result of the two photoinduced charges. As exchange interactions depend strongly on the precise geometry of the orbitals, a slight change in configuration of the pigments might have a large effect on the rate of electron (back) transfer. Alternatively, slight rearrangement may affect the width of the vibrational energy distribution function, which change may have a profound effect on the tunneling rate (D. Fredkin, personal communication).

There is a large discrepancy between the calculated effect of deuteration on the triplet yield and the experimental results. Theoretically, the triplet yield is expected to decrease from 0.17 to 0.07, experimentally no decrease is observed in cells and reaction center preparations of *R. rubrum* (17, 23). However, in the theoretical calculation we did not consider possible effects of deuteration on the rate constants for recombination. As these rate constants depend on the vibrational states of the molecules, they are expected to change upon deuterium substitution, which change may decrease the effect of the lesser value of  $A$ . Furthermore, the deuteration experiments were performed in *R. rubrum*, where in addition to the radical pair mechanism other nonintersystem crossing processes seem to be responsible for triplet formation (17). In this bacterium, an antenna carotenoid triplet is formed with high yield (0.3) upon excitation of the carotenoid, which yield is strongly decreased by a magnetic field ( $\Delta P_T/P_T \approx -0.45$ ). In the state  $P^+ I^- X^-$ , upon Bchl excitation the magnetic field effect is much lower ( $\Delta P_T/P_T \approx -0.10$ ), but it has the same dependence on the magnetic field strength as the antenna carotenoid triplet formed on carotenoid excitation. The effect of a magnetic field on Bchl-emission in *R. rubrum* resembles that in *Rps. sphaeroides*, but it is not correlated with the magnetic field-induced change in carotenoid triplet yield (33). All these observations indicate that we are not allowed to compare experimental results of *R. rubrum* with the theoretical calculations for *Rps. sphaeroides*, and that experiments on *Rps. sphaeroides* are needed to check the theory.

One reason for our inability to obtain good quantitative values for the magnetic field induced fluorescence and triplet yield changes may be that the single nuclear spin description



for the singlet-to-triplet transition is too simple. We also fitted the (zero magnetic field) experimental temperature dependence of emission and triplet yield with the high field formula of Haberkorn and Michel-Beyerle (Eq. 26). The differences between zero-field experimental values and high-field experimental values do not exceed 2.5% for the fluorescence yield and 5% for the triplet yield (5). The same standard deviations were obtained, with best-fit values of the rate constants  $\sim 40\%$  lower than when we used the low-field formula (Eq. 25), while the activation energies remained the same. Thus, the parameters resulting from the fit do not strongly depend on the functional form of the relations of Eqs. 25 and 26 and lie within the range set by the different temperature dependences of the recombination rate constants. It is clear, however, that the effect of a magnetic field is very sensitive to this functional form. Our model gives a good qualitative description of the magnetic field effect and we hope that future experimentation in this field will resolve the discrepancies between theory and experiment.

We believe that the model presented in this communication gives a consistent description of the primary events in intact cells and chromatophores of *Rps. sphaeroides*. An extension to preparations of purified reaction centers, however, may not be straightforward. Isolated reaction centers have a much lower than expected fluorescence yield when the traps are closed (34), indicating that additional deexcitation routes are present which are not incorporated in the present model. This may explain certain anomalies in the temperature dependence of delayed fluorescence and its decay kinetics that have been recently observed for reaction center preparations of *Rps. sphaeroides* R 26 (W. W. Parson, personal communication).

#### LIST OF SYMBOLS

$A$	hyperfine splitting constant of the radical pair $P^+ I^-$
Bchl	Bacteriochlorophyll
$I$	primary electron acceptor, Bacteriopheophytin
$i$	subscript, denoting nuclear spin state of the radical pair
$J$	exchange parameter
$P$	primary electron donor, Bchl-dimer
$R$	fractional concentration of reaction centers
$S$	superscript for singlet state
$T$	superscript for triplet state
$X$	secondary electron acceptor, iron-ubiquione complex

#### Rate Constants and Related Quantities

$k_c$	charge separation
$k_f$	fluorescence
$k_g$	recombination of $P^+ I^-$ to ground state
$E_g$	activation energy of this reaction
$C_g$	frequency factor of this reaction
$k_{ic}$	internal conversion
$k_{isc}$	intersystem crossing
$k_l$	energy loss; $k_l = k_f + k_{ic} + k_{isc}$
$k_r$	reduction of $X$ by $I^-$
$k_s$	recombination of $P^+ I^-$ to singlet excited state
$\Delta E$	activation energy of this reaction
$k_{so}$	frequency factor of this reaction
$k_t$	recombination of $P^+ I^-$ to reaction center triplet state

$E_i$  activation energy of this reaction  
 $C_i$  frequency factor of this reaction

### Kinetics

$f$  total kinetics of  $P^+I^-$   
 $x$  total kinetics of excited Bchl  
 $y$  single-turnover kinetics of  $P^+I^-$

### Lifetimes

$\tau'_i$  single-turnover lifetime of  $(P^+I^-)_i$   
 $\tau'$  average single-turnover lifetime of  $P^+I^-$ ;  $\tau' = \sum_i p_i \tau'_i$   
 $\tau_{rp}$  observed decay time of  $P^+I^-$   
 $\tau_{dl}$  observed decay time of delayed Bchl-emission

### Probabilities

$\phi_{si}$  single-turnover probability for formation of  $P^*I$  from  $(P^+I^-)_i$   
 $\phi_s$  average single-turnover probability for formation of  $P^*I$  from  $P^+I^-$ ;  $\phi_s = \sum_i p_i \phi_{si}$   
 $\phi_{so}$   $\phi_i$  in the state  $P I X$  (open reaction centers)  
 $\phi_{ti}$  single-turnover probability for formation  $P^T I$  from  $(P^+I^-)_i$   
 $\phi_t$  average single-turnover probability for formation of  $P^T I$  from  $P^+I^-$ ;  $\phi_t = \sum_i p_i \phi_{ti}$   
 $\phi_x$  single-turnover probability for the reaction  $P^+I^- X \rightarrow P^* I X^-$   
 $P_F$  emission yield, i.e., total probability that a photon is emitted, starting from Bchl\*  
 $P_{Fo}$   $P_F$  when all reaction centers are in state  $P I X$   
 $P_T^R$  reaction center triplet yield, i.e., the probability that a reaction center Bchl-triplet is formed, starting from Bchl\*  
 $P_T$  total triplet yield (reaction center triplet plus triplet formed by intersystem crossing)  
 $P_X$  quantum yield of photochemistry, i.e., the probability that  $X^-$  is formed, starting from Bchl\*  
 $p_i$  probability that a radical pair is in nuclear spin state  $i$

We are indebted to Dr. R. van Grondelle for stimulating discussions and to Dr. W. W. Parson for informing us of his results on bacterial reaction centers. This research was financially supported by the Netherlands Organization for the Advancement of Pure Research (ZWO) via the Foundation for Biophysics (SvB) and by the Commission of the European Communities, Energy R & D programme, Objective: Solar Energy, contract 027-76-ESN.

Received for publication 28 August 1979 and in revised form 7 January 1981.

### REFERENCES

1. Clayton, R. K. 1978. Physicochemical mechanisms in reaction centers of photosynthetic bacteria. In *The Photosynthetic Bacteria*. R. K. Clayton and W. R. Sistrom, editors. Plenum Press, New York. 387-396.
2. Hopfield, J. 1974. Electron transfer between biological molecules by thermally activated tunneling. *Proc. Natl. Acad. Sci. U.S.A.* 71:3640-3644.
3. Van Grondelle, R., N. G. Holmes, H. Rademaker, and L. N. M. Duysens. 1978. Bacteriochlorophyll fluorescence of purple bacteria at low redox potentials. The relationship between reaction center triplet yield and the emission yield. *Biochim. Biophys. Acta.* 503:10-25.
4. Shuvalov, V. A., and V. V. Klimov. 1976. The primary photoreactions in the complex cytochrome-P890-P760 (bacteriopheophytin 760) of *Chromatium minutissimum* at low redox potentials. *Biochim. Biophys. Acta.* 440:587-599.
5. Rademaker, H., A. J. Hoff, and L. N. M. Duysens. 1979. Magnetic field-induced increase of the yield of (bacterio) chlorophyll emission of some photosynthetic bacteria and of *Chlorella vulgaris*. *Biochim. Biophys. Acta.* 546:248-255.
6. Adrian, F. J. 1977. Radical pair mechanism of chemically induced magnetic polarization. In *Chemically Induced Magnetic Polarization*. L. T. Muus, P. W. Atkins, K. A. McLaughlan, and J. B. Pedersen, editors. Reidel Publishing Co., Dordrecht, The Netherlands. 77-105.

7. Rijgersberg, C. P. 1980. Fluorescence and photochemistry at low temperatures in photosynthetic systems. Ph.D. thesis, University of Leiden, The Netherlands.
8. Shipman, L. L. 1980. A theoretical study of excitations in chlorophyll *a* photosystems on a picosecond timescale. *Photochem. Photobiol.* 31:157–167.
9. Duysens, L. N. M. 1979. Transfer and trapping of excitation energy in photosystem II. In *Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Found. Symp.* 61:323–340.
10. Haberkorn, R., and M. E. Michel-Beyerle. 1979. On the mechanism of magnetic field effects in bacterial photosynthesis. *Biophys. J.* 26:489–498.
11. Hoff, A. J., H. Rademaker, R. van Grondelle, and L. N. M. Duysens. 1977. On the magnetic field dependence of the yield of the triplet state in reaction centers of photosynthetic bacteria. *Biochim. Biophys. Acta.* 460:547–554.
12. Campillo, A. J., R. C. Hyer, T. G. Monger, W. W. Parson, and S. L. Shapiro. 1977. Light collection and harvesting processes in bacterial photosynthesis investigated on a picosecond timescale. *Proc. Natl. Acad. Sci. U.S.A.* 74:1997–2001.
13. Parson, W. W., C. C. Schenck, R. E. Blankenship, D. Holten, M. W. Windsor, and C. V. Shank. 1978. Kinetics of photochemical electron transfer reactions *in vivo* and *in vitro*. In *Frontiers of Biological Energetics. Electrons to Tissues*. P. L. Dutton, J. S. Leigh, and A. Scarpa, editors. Academic Press, Inc., New York. 1:37–44.
14. Cogdell, R. J., and J. P. Thornber. 1979. The preparation and characterization of different types of light-harvesting pigment-protein complexes from some purple bacteria. In *Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Found. Symp.* 61:61–79.
15. Zankel, K. L., D. W. Reed, and R. K. Clayton. 1968. Fluorescence and photochemical quenching in photosynthetic reaction centers. *Proc. Natl. Acad. Sci. U.S.A.* 61:1243–1255.
16. Connolly, N. Y., D. S. Gorman, and G. R. Seely. 1973. Laser flash photolysis studies of chlorin and porphyrin systems. I. Energetics of the triplet state of bacteriochlorophyll. *Ann. N.Y. Acad. Sci.* 206:649–669.
17. Rademaker, H., A. J. Hoff, R. van Grondelle, and L. N. M. Duysens. 1980. Carotenoid triplet yields in normal and deuterated *Rhodospirillum rubrum*. *Biochim. Biophys. Acta.* 592:240–257.
18. Peters, K., Ph. Avouris, and P. M. Rentzepis. 1978. Picosecond dynamics of primary electron transfer processes in bacterial photosynthesis. *Biophys. J.* 23:207–217.
19. McElroy, J. D., G. Feher, and D. C. Mauzerall. 1972. Characterization of primary reactants in bacterial photosynthesis. I. Comparison of the light-induced EPR signal ( $g = 2.0026$ ) with that of a bacteriochlorophyll radical. *Biochim. Biophys. Acta.* 267:363–374.
20. Hoff, A. J., and P. Gast. 1979. Transfer of light-induced electron spin polarization in bacterial photosynthetic reaction centers. *J. Phys. Chem.* 83:3355–3358.
21. Parson, W. W., and T. G. Monger. 1977. Interrelationships among excited states in bacterial reaction centers. *Brookhaven Symp. Biol.* 28:195–212.
22. Parson, W. W., R. K. Clayton, and R. J. Cogdell. 1975. Excited states of photosynthetic reaction centers at low redox potentials. *Biochim. Biophys. Acta.* 387:265–278.
23. Blankenship, R. E., and W. W. Parson. 1979. Triplet quantum yields and decay kinetics in deuterated reaction centers from *Rhodospirillum rubrum*. *Biophys. J.* 25(2, pt. 2):205 a. (Abstr.).
24. Voznyak, V. M., E. I. Elfimov, and V. R. Suskavatzina. 1980. Magnetic field affects the fluorescence yield in reaction center preparations from *Rhodopseudomonas sphaeroides* R-26. *Biochim. Biophys. Acta.* 592:235–239.
25. Van Bochove, A. C., R. van Grondelle, and L. N. M. Duysens. 1980. Nanosecond recombination luminescence of bacteriochlorophyll chromatophores of various photosynthetic purple bacteria. Proceedings Fifth International Congress on Photosynthesis. Halkidiki. In press.
26. Sonneveld, A., L. N. M. Duysens, and A. Moerdijk. 1980. Magnetic field-induced increase in chlorophyll *a* delayed fluorescence of photosystem II: A 100- to 200-ns component between 4.2 and 300 K. *Proc. Natl. Acad. Sci. U.S.A.* 77:5889–5893.
27. Godik, V. I., and A. Y. Borisov. 1979. Short-lived delayed luminescence of photosynthetic organisms. I. Nanosecond afterglows in purple bacteria at low redox potential. *Biochim. Biophys. Acta.* 548:296–308.
28. Seely, G. R. 1978. The energetics of electron-transfer reactions of chlorophyll and other compounds. *Photochem. Photobiol.* 27:639–654.
29. Fajer, J., D. C. Brune, M. S. Davis, A. Forman, and L. D. Spaulding. 1975. Primary charge separation in bacterial photosynthesis: oxidized chlorophylls and reduced pheophytin. *Proc. Natl. Acad. Sci. U.S.A.* 72:4956–4960.
30. Seely, G. R. 1978. Photochemistry of chlorophyll in solutions: modelling photosystem II. *Curr. Top. Bioenerg.* 7:3–37.

31. Cotton, T. M., and R. P. van Duyne. 1979. An electrochemical investigation of the redox properties of bacteriochlorophyll and bacteriopheophytin in aprotic solvents. *J. Am. Chem. Soc.* 101:7605-7612.
32. Blankenship, R. E. and W. W. Parson. 1979. Kinetics and thermodynamics of electron transfer in bacterial reaction centers. *In* Photosynthesis in Relation to Model Systems. Topics in Photosynthesis. Vol. 3. J. Barber, editor. Elsevier North-Holland Biomedical Press, Amsterdam. 71-114.
33. Rademaker, H. 1980. Magnetic field effects on primary and associated reactions of bacterial photosynthesis. Ph.D. Thesis, University of Leiden, Netherlands.
34. Slooten, L. 1972. Reaction center preparations of *Rhodopseudomonas sphaeroides*: energy transfer and structure. *Biochim. Biophys. Acta.* 256:452-466.