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FREE ENERGY CHANGE ACCOMPANYING ELECTRON TRANSFER FROM P_{870} TO QUINONE IN *RHODOPSEUDOMONAS SPHAEROIDES* CHROMATOPHORES

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Delayed fluorescence of chromatophores of *Rhodopseudomonas sphaeroides* was measured to estimate the standard free energy change accompanying the electron transfer from the bacteriochlorophyll dimer (P) to the primary acceptor quinone (Q_A). The chromatophores emitted delayed fluorescence with a lifetime of about 60 ms in the presence of *o*-phenanthroline. By comparing the intensity of the delayed fluorescence with that of the prompt fluorescence, the standard free energy of the $P^+Q_A^-$ radical pair was evaluated. It was about 0.87 eV below the level of excited singlet state, P^*Q_A, or 0.51 eV above the ground state, PQ_A, independent of pH.

Introduction

In bacterial photosynthesis, the primary photochemical reaction takes place in a pigment-protein complex called the reaction center [1,2]. A bacteriochlorophyll dimer (P_{860} or P), raised to an excited singlet state by light, transfers its electron to a primary acceptor quinone molecule (Q_A) via bacteriopheophytin [1–4] and, probably, via one of the other bacteriochlorophyll molecules in the reaction center complex, P_{800} [5]. Q_A^- passes the electron to a second quinone molecule (Q_B) in about 100 μs [6].

Under normal conditions, Q_A is reduced only to the level of semiquinone. The midpoint redox potential (E_m) of Q_A^-/Q_A couple has been measured by monitoring the photochemical activity or ESR signal of the semiquinone anion radical as a function of ambient redox potential [7,8]. It decreased linearly with increasing pH, indicating that the uptake of the proton was coupled to the reduction [7,8]. The apparent pK of Q_A^- depended on bacterial species. In *Rhodopseudomonas sphaeroides,* it was about 10; above this pH, the E_m was about -180 mV, independent of pH [9]. Since no proton uptake was observed after the light-induced reduction of Q_A, the functional E_m of Q_A is thought to be -180 mV [9]. The E_m of P is about 450 mV in *R. sphaeroides* [10,11]. Thus, a radical pair $P^+Q_A^-$ has a standard free energy of 0.63 eV relative to the ground state, PQ_A.

This value is, however, subject to some criticism [4]: first, we have no information on the magnitude of the interaction between P^+ and Q_A^-; second, the final equilibration state in the titration of E_m is not necessarily the same as the transient state that appears in the light-induced electron transfer.

Arata and Parson [12] estimated the standard free energy of the $P^+Q_A^-$ pair in the isolated reaction center by measuring delayed fluorescence. It was 0.52 ± 0.02 eV above PQ_A. However, it is doubtful that this value is applicable to the intact chromatophore membrane, because the E_m of Q_A in isolated reaction centers was different from that in chromatophores, where it was -50 mV, independent of pH in the detergent-solublized state [13,14]. In phospholipid vesicles, the E_m depended

on pH, amount of ubiquinone added, and *o*-phenanthroline [15].

In the present study, we applied the method of delayed fluorescence [12] to chromatophores. The value we obtained was about 0.51 eV, which was close to the value measured in isolated reaction centers, but not to the difference of the two E_m values titrated with chromatophore membranes.

Materials and Methods

Cells of *Rhodopseudomonas sphaeroides* strain 2.4.1 (wild type) were cultured in the medium described by Cohen-Bazire et al. [16]. Chromatophores free of cytochrome c_2 were prepared as follows. Spheroplasts were prepared by a method basically the same as that of Witholt et al. [17] (see also Ref. 18), and washed once with 10 mM Tris-HCl (pH 8.0)/1 M KCl/5 mM $MgCl_2$. The spheroplasts were then passed through a French pressure cell (1000 kg/cm^2) and centrifuged at $10\,000 \times g$ for 20 min. Chromatophores in the supernatant were collected by centrifugation at $120\,000 \times g$ for 60 min, washed with and resuspended in a buffer comprising 10 mM Tris-HCl/(pH 8.0)/100 mM KCl.

For the measurements of delayed fluorescence, the samples were placed in a 1×1 cm plastic cuvette in a thermostatically controlled housing. A 1 μs xenon flash (MF-502-UI, Sugawara Laboratories) was passed through a Corning-9782 glass filter or a 585 nm interference filter, and guided to the two opposing sides of the sample cuvette by glass-fiber tubes. Fluorescence was measured at 90° to the excitation beams, with a cooled 7102 (S1-type) photomultiplier covered with a Corning-2600 glass filter and a mechanical shutter. The shutter gave a signal to trigger the flash at about 10 ms before it opened. The photomultiplier signal was amplified and sent to a transient recorder (Riken Denshi, TCC-1000S) and an averager (Riken Denshi, ITC-4). All the measurements were made at 311 K. The chromatophores were stable at this temperature during the measurements (for more than 1 h). We could not detect the delayed fluorescence at room temperature.

Oxidation-reduction time-courses of P were recorded by measuring the absorbance changes at 605 nm. The samples were excited by a xenon flash (MF-1500-U3, Sugawara Laboratories) passed through a Wratten-88A filter.

The quantum yield of fluorescence of the chromatophores was measured by comparison with Rhodamine B fluorescence, which has a quantum yield of 97% in ethanol [19]. The chromatophores and the Rhodamine B solution were excited by the same flash as used for the delayed fluorescence measurements. A 585 nm interference filter was used in conjunction with a Corning-9788 filter and neutral density filters for excitation of the chromatophores. For Rhodamine B excitation, the 585 nm interference filter was replaced by a 510 nm interference filter. The absorbances of the chromatophore suspension and the Rhodamine B solution were adjusted to about 0.09 at the respective wavelengths. Since the interference filters used had rather broad transmission bands, the fractions of photons absorbed were calculated using the transmission spectra of the filters and the absorption spectra of the samples. The intensity of the exciting flash was measured by a calibrated thermopile. The photomultiplier (7102) was covered by a Corning-2600 filter for the chromatophores, and by a Toshiba V-O2 filter (540 nm cut-off filter) for Rhodamine B. Difference in the quantum efficiency of the detector systems for the fluorescences was computed from the fluorescence spectra, the transmission spectra of the filters, and the quantum efficiency spectrum of the photomultiplier.

The fluorescence spectra of the chromatophores were measured with continuous light from a tungsten-halogen lamp passed through a Corning-9782 filter. The spectrum of the initial level of fluorescence at the onset of the illumination was used for the calculation of the efficiency of the detector system. The fluorescence spectrum of Rhodamine B was measured with 470 nm continuous light obtained with the tungsten-halogen lamp and an interference filter. The quantum efficiency of the photomultiplier was calibrated as a function of wavelength, using monochromatic continuous light, intensity of which was measured by a calibrated thermopile.

Concentration of bacteriochlorophyll in the chromatophore preparation was measured from absorbance at 770 nm of an acetone-methanol extract using an absorption coefficient of 75 mM^{-1}

cm^{-1} [20]. The concentration of P was determined by flash-induced absorbance change at 605 nm using a difference absorption coefficient of 19 $mM^{-1}\,cm^{-1}$ [21].

Results

Fig. 1 shows typical time-courses of delayed fluorescence and re-reduction of P^+ after the flash excitation. In the absence of *o*-phenanthroline, which blocks the electron transfer from Q_A to Q_B [22,23], P^+ decayed slowly (trace D), since the electron donor, cytochrome c_2, was removed by washing. No fluorescence with a lifetime longer than 10 ms was detected (trace A). In the presence of 2 mM *o*-phenanthroline, P^+ was reduced with a lifetime of about 60 ms (trace E) by electrons on Q_A. Part of P^+ (10–20%) decayed slowly, probably because the inhibition by *o*-phenanthroline was not complete [24]. Delayed fluorescence with a similar lifetime was observed in the presence of the inhibitor (trace B). In some preparations, the decay of fluorescence was somewhat faster than the re-reduction of P^+, as observed with isolated reaction centers [12].

A basic assumption needed for estimating the standard free energy of the state $P^+Q_A^-$ from the intensity of the delayed fluorescence is that the photochemical reaction:

$$P^*Q_A \rightleftarrows P^+Q_A^- \qquad (1)$$

reaches equilibrium rapidly (in about 200 ps [4]) after the flash excitation, and stays in equilibrium during the lifetime of the state $P^+Q_A^-$ [12]. Here, P* represents the excited singlet state of P. The concentration of P* after the equilibration is:

$$[P^*] = K[P^+Q_A^-] = \exp\left(-\frac{\Delta G_0}{kT}\right)[P^+Q_A^-] \qquad (2)$$

where K and ΔG_0 are the equilibrium constant and the change in standard partial molecular free energy, respectively, of the reaction shown in Eqn. 1, and k is the Boltzmann constant.

In isolated reaction centers, P* is the only molecular species that emits delayed fluorescence: the chance of excitation transfer from P_{870} to P_{800} would be small because of the large energy gap. In chromatophores, the excitation would be transferred to antenna bacteriochlorophyll molecules and the delayed fluorescence is mostly emitted from them [24,25].

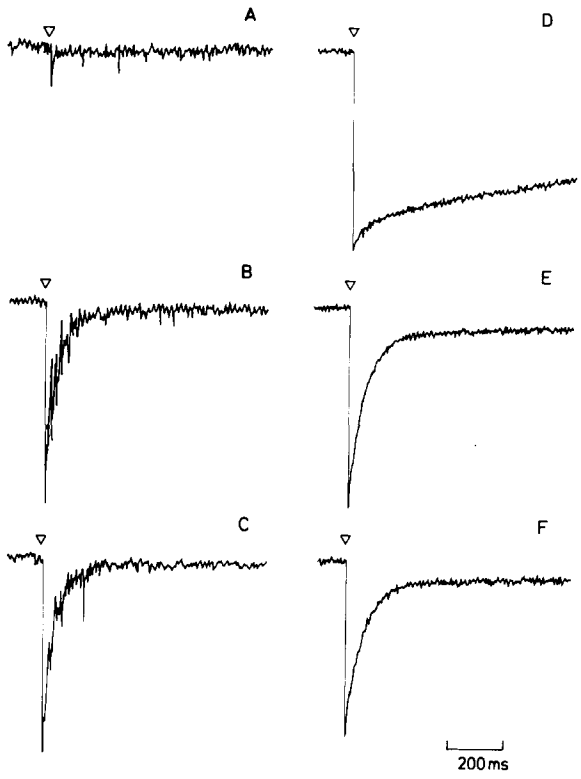

Fig. 1. Time-courses of delayed fluorescence and absorption change at 605 nm. Traces A, B and C, delayed fluorescence. The shutter in front of the photomultiplier was opened 10 ms after the flash excitation. Traces D, E and F, absorbance change at 605 nm. The downward deflection corresponds to the decrease of absorbance. Cytochrome c_2-free chromatophores (35 μM bacteriochlorophyll) were suspended in 10 mM Tris-HCl (pH 8.0)/100 mM KCl. (A) and (D), no further addition; (B) and (E), 2 mM *o*-phenanthroline was added; (C) and (F), 2 mM *o*-phenanthroline and 2 μg/ml gramicidin D were added. Intensity of the flash, 0.1 $mJ \cdot cm^{-2}$. Signals were averaged 16 times for the delayed fluorescence and four times for the absorbance change.

If the energy transfer between antenna bacteriochlorophyll species is rapid relative to radiative and non-radiative loss of the excitation energy, we can suppose a thermal equilibrium in the distribution of the excitation:

$$[B_i^*] = N_i \exp\left(-\frac{\mu_i^* - \mu_P^*}{kT}\right)[P^*] \qquad (3)$$

where B_i^* is the singlet excited state of the ith component of the antenna bacteriochlorophyll forms, B_i, N_i is the number of B_i per reaction center, and μ_i^* and μ_P^* are the standard molecular free energies of the singlet excited states of B_i and P, respectively, relative to that of the ground state. If B* and P* fluoresce with rate constants of k_i^F and k_P^F, the intensity of the delayed fluorescence will be:

$$F_d = k^F K [P^+ Q_A^-] \tag{4}$$

$$\int_0^\infty F_d(t)\,dt = \frac{k^F}{k_d} K [P^+ Q_A^-]_0 \tag{5}$$

where

$$k^F \equiv k_P^F + \sum_i k_i^F N_i \exp\left(-\frac{\mu_i^* - \mu_P^*}{kT}\right) \tag{6}$$

t is the time after the flash excitation and k_d is the first-order rate constant on the decay of the delayed fluorescence. $[P^+Q_A^-]_0$ represents the concentration of $P^+Q_A^-$ formed by the flash.

In order to calculate K, the intensity of the delayed fluorescence should be compared with fluorescence of a known quantum yield. We used 'prompt' fluorescence of the same sample as the standard, because a possible problem caused by re-absorption of the emitted light would be minimized. The ratio of the delayed to the total fluorescence is:

$$\frac{\int_0^\infty F_d(t)\,dt}{\int_{-\infty}^\infty F(t)\,dt} = \frac{k^F K \phi_A}{k_d \phi_F} \tag{7}$$

where ϕ_A and ϕ_F are the quantum yields of the $P^+Q_A^-$ formation and the fluorescence, respectively. The delayed fluorescence was measured with a flash passed through the 585 nm interference filter. The intensity of the flash ($1.8 \cdot 10^{-6}$ J · cm^{-2}) was sufficiently weak that the delayed fluorescence was proportional to the intensity. $\int F_d\,dt$ was calculated from a part of the integral of the time-course of the delayed fluorescence, using k_d obtained with a stronger flash with a Corning-9782 filter. The decay did not depend on the flash intensity when the Corning-9782 filter was used (Fig. 2). The total fluorescence $\int F\,dt$ was measured with the shutter kept open. The flash light was passed through a Corning-9788 filter and appropriate neutral density filters besides the interference filter, so that the fluorescence could be measured with the same detector system. The apparatus did not have sufficient time resolution to measure the time-course of the fluorescence elicited by the 1 μs exciting flash. But the integration of time courses measured by the same detector system gave the same proportionality in both the

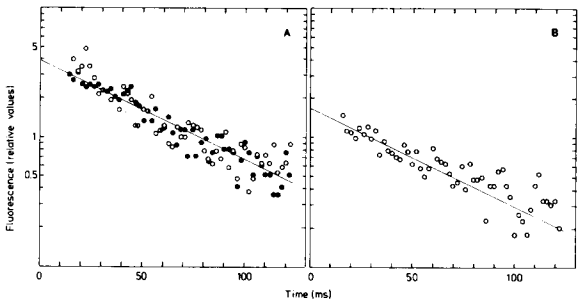

Fig. 2. Time-courses of delayed fluorescence plotted on a semilogarithmic scale. Chromatophores (35 μM bacteriochlorophyll) were suspended in 10 mM Tris-HCl (pH 8.0)/100 mM KCl. 2 mM *o*-phenanthroline present. ○, no further addition; ●, 2 μg/ml gramicidin D. Intensity of the flash, 0.1 mJ·cm^{-2} (A) or 0.04 mJ·cm^{-2} (B). Signals were averaged 16 times for A and 32 times for B. The solid lines were drawn supposing the same first-order rate constant (17.7 s^{-1}) as that of the fast component in the re-reduction of P^+.

TABLE I

EVALUATION OF THE EQUILIBRIUM CONSTANT AND THE STANDARD PARTIAL MOLECULAR FREE ENERGY CHANGE FOR THE ELECTRON TRANSFER FROM P* TO Q_A

For the definition and the methods of measurements of the parameters $\int F_d\,dt/\int F\,dt$, ϕ_F and k^F, see the text. Results with three different preparations are listed.

Preparation	$\int F_d\,dt/\int F\,dt$ ($\times 10^5$)	ϕ_F ($\times 10^3$)	k^F (s^{-1}) ($\times 10^{-8}$)	K ($\times 10^{14}$)	ΔG_0 (eV)
1	2.7	8.4	12.7	0.45	−0.885
2	5.1	5.5	6.4	1.29	−0.857
3	5.1	7.6	8.9	1.07	−0.862
Mean					−0868 ±0.015

prompt and delayed fluorescence. The intensity of the fluorescence was proportional to the flash strength in the range we used. The value of $\int F_d\,dt/\int F\,dt$ was $3\text{–}5 \cdot 10^{-5}$ (Table I).

A possible problem in the estimation of the standard free energy of $P^+Q_A^-$ in the chromatophore membrane is an effect of the electric field generated by the simultaneous vectorial movement of electrons from P to Q_A. The electric field would intensify the delayed fluorescence [26,27] and would cause an underestimation of ΔG_0. However, we think that the effect is negligible for the following reasons. First, gramicidin D did not affect the intensity and the decay kinetics of the delayed fluorescence significantly (Fig. 1, trace C; Fig. 2, closed circles). Second, the decay kinetics did not depend on the intensity of the flash (Fig. 2). Since the fluorescence would depend exponentially on the electrical potential difference between the positions of P and Q_A, the decay of the delayed fluorescence would not follow first-order kinetics and would become faster with an increase in the charge separation [27]. This was not observed, probably because the flash was weak. In the estimation of K, the intensity of the exciting flash was much weaker than that used in Fig. 2.

The value of k^F, the rate constant of the total radiative loss of the excitation, as defined by Eqn. 6, can be evaluated using the fluorescence spectrum (see Appendix). The spectrum of the variable part of the fluorescence excited by continuous light was used for the estimation, for it represented the equilibrium fluorescence [25,28] and was the same as the spectrum of the delayed fluorescence [25]. The cross-section of the 'photosynthetic unit' was measured from the absorbance change at 605 nm by a saturating flash. The refractive index was supposed to be 1.5 and Z/Z^*, the ratio of the partition functions of P and P*, defined with respect to the lowest vibrational levels of the two states, was supposed to be 1. Although we do not know the true value of Z/Z^*, the possible error is cancelled in the estimation of the standard free energy relative to the ground state [12].

The fluorescence yield, ϕ_F, was determined by reference to Rhodamine B, the fluorescence yield of which is known. That fraction of the P^+ signal which decayed with the larger rate constant was used for ϕ_A. (The quantum yield of the $P^+Q_A^-$ formation is taken as unity.) $P^+Q_A^-$ in the uninhibited reaction centers relaxed to $P^+Q_AQ_B^-$ in about 100 μs [4], and the corresponding delayed fluorescence was not measured. Table I summarizes the values of $\int F_d\,dt/\int F\,dt$, ϕ_F and k^F measured with three different preparations, and K and ΔG_0 calculated. The mean value of ΔG_0 in three different preparations was 0.868 ± 0.015 eV. Since the standard molecular free energy for the excitation of P is 1.38 eV, $P^+Q_A^-$ was about 0.51 eV above the ground state in the standard free energy.

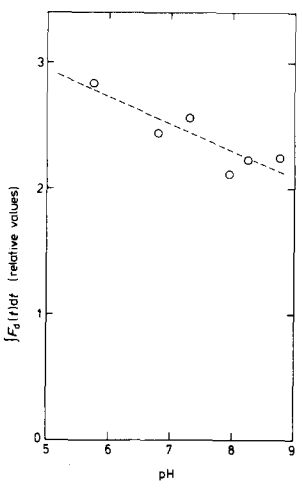

Fig. 3. Dependence of the delayed fluorescence on pH of the medium. The experimental conditions were as in Fig. 1. 2 mM *o*-phenanthroline present.

Fig. 3 shows the intensity of the delayed fluorescence as a function of pH of the medium. Integrated values are plotted in the figure. The decay did not depend on pH. The intensity slightly decreased with increasing pH. The fast component in the re-reduction kinetics of P^+ also decreased with increasing pH (data not shown), probably because the effect of *o*-phenanthroline depended on pH. Thus, the standard free energy of $P^+Q_A^-$ is independent of pH.

Discussion

The standard free energy of the $P^+Q_A^-$ radical pair was calculated to be 0.51 eV above the ground state, essentially independent of pH. This value agrees well with the value measured in isolated

reaction centers [12]. The agreement could, however, be fortuitous, because the thermodynamic properties of Q_A may be altered significantly when reaction centers are solubilized and isolated [13,14]. The value is, on the other hand, significantly smaller than the difference between the E_m values of P and Q_A (0.63 eV). If we assume that the discrepancy is simply due to the interaction of P^+ and Q_A^-, the standard free energy change for dismutation:

$$P^+Q_A + PQ_A^- \rightleftarrows P^+Q_A^- + PQ_A \quad (8)$$

is 0.12 eV. The interaction energy would be bigger if any additional relaxation takes place in the titration of the E_m. The value 0.12 eV is not surprisingly large: the Coulombic interaction energy between two elementary charges that are 20 Å apart is about 0.72 eV in vacuo.

In the isolated reaction center, the interaction was apparently small [12]. This contrast could arise from the difference environment: the reaction center is exposed to water in the isolated state, while it is embedded in membrane dielectrics in chromatophores.

Another possible origin of the discrepancy between the value estimated here and the difference of the titrated E_m values is interaction with other redox components. For example, the E_m of Q_A could depend on the redox state of Q_B. In the redox titration of Q_A, most Q_B may exist in the fully reduced state, while it is oxidized when the state $P^+Q_A^-$ is formed by a flash. The E_m of the $Q_A^-Q_B/Q_AQ_B$ couple could be different from that of the $Q_A^-Q_BH_2/Q_AQ_BH_2$ couple. We have no evidence that this is indeed the case, but we do have evidence that the E_m of Q_A depends on the state of the quinone on the Q_B site. First, the state $P^+Q_A^-Q_B^-$ has a higher standard free energy than the state $P^+Q_A^-Q_B$. Carithers and Parson [24] measured delayed fluorescence with a lifetime of 120 μs. It was weak after the first flash and was enhanced by repetitive flashes. Vermeglio et al. [29] suggested that the electrons on Q_A are transferred to Q_B slowly after the flash even in the presence of *o*-phenanthroline, and that the subsequent flash forms the $P^+Q_A^-Q_B^-$ state, which would cause stronger fluorescence. Second, the E_m of Q_A depends on the presence of the quinol on the Q_B site [15]. It became higher in reaction centers that have only one quinone and binding of an added quinone molecule (actually QH_2) to the Q_B site reversed this effect.

We measured the delayed fluorescence in the presence of *o*-phenanthroline. Wraight [15] has suggested that *o*-phenanthroline replaced Q_B. If he is correct, the value we obtained is the standard free energy of $P^+Q_A^-$ without ubiquinone at the Q_B site and could be different from that of $P^+Q_A^-Q_B$. *o*-Phenanthroline increased the E_m of Q_A by about 40 mV in chromatophores of *R. sphaeroides* [11], although the E_m has not been determined at a pH above the pK of Q_A^- in the presence of *o*-phenanthroline. In other bacterial species [9] and isolated reaction centers in phospholipid vesicles [15], *o*-phenanthroline did not change the E_m of Q_A at a pH above the pK of Q_A^-. However, all of these results are from redox titrations and may not give full information for the comparison of the standard free energy of the states $P^+Q_A^-$-*o*-phenanthroline and $P^+Q_A^-Q_B$.

We did not detect delayed fluorescence in the absence of *o*-phenanthroline, when the pH was less than 10. This is probably because relatively small free energy is stored when the electron is passed to Q_B or further. Rutherford and Evans [8] titrated the E_m of Q_B^-/Q_B and Q_BH_2/Q_B^- couples by EPR spectroscopy. The E_m for the reduction to the semiquinone was about 40 mV at pH 8. This value gives 0.41 eV for the standard free energy of the state $P^+Q_AQ_B^-$ if we neglect the interaction between P^+ and Q_B^-. The delayed fluorescence with no inhibitors would be only 1/50 of that in the presence of *o*-phenanthroline. If electrons can go further rapidly, it would be even weaker. Although our instrument is not sensitive enough to analyze the uninhibited chromatophores, an improvement is possible and information about the free energy input to cytochrome b-c_1 complexes will help us to analyze the electron transfer coupled to the proton translocation.

Appendix

Ross [30] has summarized useful methods for evaluation of the radiative lifetime of an electronically-excited state from absorption or fluorescence spectrum. We applied the method of integration of

the fluorescence spectrum, to bacterial photosynthetic units composed of the reaction center and many components of antenna bacteriochlorophyll species.

At the thermal equilibrium in a dark enclosure, the rate of upward and downward transitions between two electronic states associated with radiation at frequency ν are equal (detailed balance) for each component i:

$$\kappa_i^{\mathrm{A}}(\nu)C_i = \kappa_i^{\mathrm{F}}(\nu)C_i^* \tag{A-1}$$

where $\kappa_i^{\mathrm{A}}(\nu)$ and $\kappa_i^{\mathrm{F}}(\nu)$ are the rate constants of the upward and downward radiative transitions at frequency ν between ground and excited states, and C_i and C_i^* are the concentrations of molecules in the lower and upper states. The rate constants of the upward transition are:

$$\kappa_i^{\mathrm{A}}(\nu) = I_{\mathrm{BB}}(\nu)\sigma_i(\nu) \tag{A-2}$$

where σ_i is the molecular absorption cross-section of i and I_{BB} is the intensity of black-body radiation given by the Planck's law:

$$I_{\mathrm{BB}}(\nu) = 8\pi\left(\frac{n\nu}{c}\right)^2\left\{\exp\left(\frac{h\nu}{kT}\right)-1\right\}^{-1} \tag{A-3}$$

Combining Eqns. A-1–A-3, we obtain:

$$\kappa_i^{\mathrm{F}}(\nu)\,\mathrm{d}\nu = \frac{C_i}{C_i^*}8\pi\left(\frac{n\nu}{c}\right)^2\exp\left(-\frac{h\nu}{kT}\right)\sigma_i(\nu)\,\mathrm{d}\nu \tag{A-4}$$

as $h\nu/kT \gg 1$.

At equilibrium, C_i/C_i^* is determined by the standard molecular free energy difference, μ_i^*, between the ground and excited states:

$$\frac{C_i}{C_i^*} = \exp\frac{\mu_i^*}{kT} = \frac{Z_i}{Z_i^*}\exp\frac{E_i^*}{kT} \tag{A-5}$$

where E_i^* is the difference between the energies of the ground vibrational levels of the ground and excited states, and Z_i and $Z^*{}_i$ are the partition functions defined with respect to the ground vibrational energy of the ground and excited states, respectively. Thus:

$$\kappa_i^{\mathrm{F}}(\nu)\,\mathrm{d}\nu = 8\pi\left(\frac{n\nu}{c}\right)^2\exp\left(\frac{\mu_i^*-h\nu}{kT}\right)\sigma_i(\nu)\,\mathrm{d}\nu \tag{A-6}$$

Eqn. A-6 applies to the radiation in the state out of equilibrium and gives the fluorescence spectrum of the ith component.

If equilibrium is established in the excitation transfer among the pigment components, the rate constant $\kappa^{\mathrm{F}}(\nu)$ for the total radiative downward transitions is:

$$\begin{aligned}\kappa^{\mathrm{F}}(\nu)\,\mathrm{d}\nu &= \sum_i \kappa_i^{\mathrm{F}}(\nu)\exp\left(-\frac{\mu_i^*-\mu_{\mathrm{P}}^*}{kT}\right)N_i\,\mathrm{d}\nu \\ &= 8\pi\left(\frac{n\nu}{c}\right)^2\exp\left(\frac{\mu_{\mathrm{P}}^*-h\nu}{kT}\right)\sum_i\sigma_i(\nu)N_i\,\mathrm{d}\nu \\ &= 8\pi\left(\frac{n\nu}{c}\right)^2\exp\left(\frac{\mu_{\mathrm{P}}^*-h\nu}{kT}\right)\sigma(\nu)\,\mathrm{d}\nu\end{aligned} \tag{A-7}$$

where N_i is the number of the ith component per reaction center, and $\sigma(\nu)$ ($\equiv \Sigma_i\sigma_i(\nu)N_i$) is the cross section of the 'photosynthetic unit'. The subscript P represents the primary electron donor in the reaction center. Since the total fluorescence has a spectrum proportional to Eqn. A-7,

$$\kappa^{\mathrm{F}}(\nu)\,\mathrm{d}\nu = \frac{Z_{\mathrm{P}}}{Z_{\mathrm{P}}^*}8\pi\left(\frac{n\nu'}{c}\right)^2\exp\left(\frac{E_{\mathrm{P}}^*-h\nu'}{kT}\right)\frac{\sigma(\nu')}{F(\nu')}F(\nu)\,\mathrm{d}\nu \tag{A-8}$$

where ν' is a frequency at which σ/F can be determined with accuracy. We now use $\nu_0 \equiv E_{\mathrm{P}}^*/h$ as the reference frequency:

$$\kappa^{\mathrm{F}}(\nu)\,\mathrm{d}\nu = \frac{Z_{\mathrm{P}}}{Z_{\mathrm{P}}^*}8\pi\left(\frac{n\nu_0}{c}\right)^2\frac{\sigma(\nu_0)}{F(\nu_0)}F(\nu)\,\mathrm{d}\nu \tag{A-9}$$

Integrating Eqn. (A-9), the rate constant for the total radiative decay is:

$$k^{\mathrm{F}} = \frac{Z_{\mathrm{P}}}{Z_{\mathrm{P}}^*}8\pi\left(\frac{n\nu_0}{c}\right)^2\frac{\sigma(\nu_0)}{F(\nu_0)}\int F(\nu)\,\mathrm{d}\nu \tag{A-10}$$

ν_0 can be determined from the reflection axis that makes the absorption and the fluorescence spectra mirror images of one another in the isolated reaction center, if E_{P}^* is not altered by the solublization and isolation procedures. The wavelength of the reflexion axis, c/ν_0, is 895 nm and corresponds to 1.38 eV. $Z_{\mathrm{P}}/Z_{\mathrm{P}}^*$ is the only unknown factor.

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References

1 Feher, G. and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 349–386, Plenum Press, New York
2 Clayton, R.K. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 387–396, Plenum Press, New York
3 Parson, W.W. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 416, 105–149
4 Blankenship, R.E. and Parson, W.W. (1979) in Photosynthesis in Relation to Model Systems (Barber, J., ed.), pp. 71–114, Elsevier, Amsterdam
5 Shuvalov, V.A. and Parson, W.W. (1980) Proc. Natl. Acad. Sci. U.S.A. 78, 957–961
6 Parson, W.W. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 455–469, Plenum Press, New York
7 Prince, R.C. and Dutton, P.L. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 439–453, Plenum Press, New York
8 Rutherford, A.W. and Evans, M.C.W. (1980) FEBS Lett. 110, 257–261
9 Prince, R.C. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 329–334
10 Dutton, P.L. and Jackson, J.B. (1972) Eur. J. Biochem. 30, 495–510
11 Jackson, J.B., Cogdell, R.C. and Crofts, A.R. (1973) Biochim. Biophys. Acta 290, 218–225
12 Arata, H. and Parson, W.W. (1981) Biochim. Biophys. Acta 638, 201–209
13 Reed, D.W., Zankel, K.L. and Clayton, R.K. (1962) Proc. Natl. Acad. Sci. U.S.A. 63, 42–46
14 Dutton, P.L., Leigh, J.S. and Wraight, C.A. (1973) FEBS Lett. 36, 169–173
15 Wraight, C.A. (1981) Isr. J. Chem. 21, 348–354
16 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1975) J. Cell Comp. Physiol. 49, 25–68
17 Witholt, B., Boebhout, M., Brock, M., Kingma, J., Van Heerikhuizen, H. and De Leij, L. (1976) Anal. Biochem. 74, 160–170
18 Hashimoto, K. and Nishimura, M. (1981) J. Biochem. 89, 909–918
19 Weber, G. and Teale, F.W.J. (1957) Trans. Faraday Soc. 53, 646–655
20 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312–323
21 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556
22 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232–245
23 Clayton, R.K., Szuts, E.Z. and Fleming, H. (1972) Biophys. J. 12, 64–79
24 Carithers, R.P. and Parson, W.W. (1976) Biochim. Biophys. Acta 440, 215–232
25 Zankel, K.L. (1969) Photochem. Photobiol. 10, 259–266
26 Fleischmann, D.E. (1971) Photochem. Photobiol. 14, 277–286
27 Arata, H., Takamiya, K. and Nishimura, M. (1977) J. Biochem. 81, 1133–1139
28 Zankel, K.L. and Clayton, R.K. (1969) Photochem. Photobiol. 9, 7–15
29 Vermeglio, A., Martinet, T. and Clayton, R.K. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1809–1813
30 Ross, R.T. (1975) Photochem. Photobiol. 21, 401–406