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MAGNETIC FIELD-INDUCED INCREASE OF THE YIELD OF (BACTERIO)CHLOROPHYLL EMISSION OF SOME PHOTOSYNTHETIC BACTERIA AND OF *CHLORELLA VULGARIS*

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Summary

In photosynthetic bacteria, in which the iron-ubiquinone complex X is prereduced, a magnetic field induces an increase of the emission yield, which is correlated with the decrease in reaction center triplet yield reported previously (Hoff, A.J., Rademaker, H., van Grondelle, R. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 460, 547—554). Our results support the hypothesis that under these conditions charge recombination of the oxidized primary donor and the reduced primary acceptor predominantly generates the excited singlet state of the reaction center bacteriochlorophyll.

In *Chlorella vulgaris* and spinach chloroplasts, at 120 K, the magnetic field has an effect similar to that found in bacteria, which suggests that an intermediary electron acceptor between *P*-680 and Q is present in Photosystem II also.

Introduction

Recently it has been found that a static magnetic field decreases the quantum yield of the photoinduced triplet state in photosynthetic bacteria in which X, the secondary acceptor (an iron-ubiquinone complex) was prereduced [1,2]. The phenomenon was explained in terms of the so-called radical pair mechanism: charge separation results in the radical pair P^+I^-, where P is the primary donor (a bacteriochlorophyll dimer) and I the primary acceptor (a bacteriopheophytin; [3—8]). The spins on P^+ and I^- oscillate between a singlet state $(P^+I^-)^S$ and a virtual triplet state $(P^+I^-)^T$ (see Ref. 9 for an introduction to

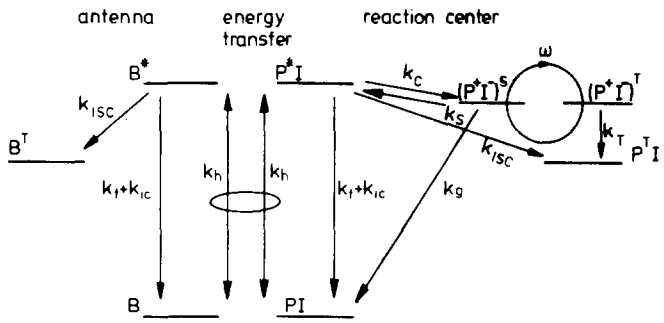

Fig. 1. Scheme for energy transfer and electron redistribution reactions occurring in the antenna bacteriochlorophyll and the reaction center, with reduced secondary acceptor X. k_h, k_f, k_{ic} and k_{isc} are the rate constants for energy transfer, fluorescence, radiationless decay and intersystem crossing to the triplet state B^T or P^T, respectively. k_c is the rate constant for charge separation, k_t, k_s and k_g are the rate constants for the recombination of P^+I^- to the triplet state, the excited singlet state and the ground state, respectively. ω is the angular frequency for interconversion between the singlet and triplet state of P^+I^-.

the theory of the radical pair mechanism). From $(P^+I^-)^T$ the charges will combine to the reaction center triplet state, P^T, whereas from $(P^+I^-)^S$ the charges may combine to either the excited singlet state P^*I or to the ground state $P\,I$. Indications for an efficient back reaction to P^*I have recently been obtained by the study of the fluorescence yield as a function of temperature in bacterial cells at low redox potentials [10,11]. The various possible reaction pathways are schematically depicted in Fig. 1.

In a magnetic field B the triplet state is composed of three sublevels, T_1, T_0 and T_{-1}, corresponding to the values of the magnetic quantum number m_s; the energy levels are separated by the Zeeman energy $g\,\beta\,B$. In a high magnetic field only T_0 is sufficiently close to the singlet level for the interconversion $(P^+I^-)^S \rightarrow (P^+I^-)^T$ to occur, so that the probability to recombine to P^TI is reduced by the presence of the field. The decrease of the triplet yield increases the concentration of $(P^+I^-)^S$ and thus the rate for recombining to the state P^*I; consequently the concentration of P^*I and the emission yield increases.

In this communication we examine the effect of a static magnetic field on the emission yield of bacteriochlorophyll of whole cells and chromatophores of the photosynthetic bacteria *Rhodopseudomonas sphaeroides* (strain 2.4.1 and its carotenoid-less mutant R-26) and *Rhodospirillum rubrum* (strain S1). We have found that in state PIX^- the emission yield is an increasing function of the strength of the magnetic field and is correlated with a decreasing yield of triplet formation. Our results support the hypothesis [11], that charge recombination predominantly generates the excited singlet state P^*I.

In *Chlorella vulgaris* and spinach chloroplasts at 120 K the curve of the yield of the fluorescence vs. the field strength, measured at 685 or 695 nm, was similar to that found for bacteria. This suggests that also in Photosystem II an intermediary electron acceptor is present between *P*-680 and Q.

Materials and Methods

Cells of *Rps. sphaeroides*, *R. rubrum* and *Cl. vulgaris* were grown as described earlier [12,13]. The suspensions of algae and bacteria were taken as

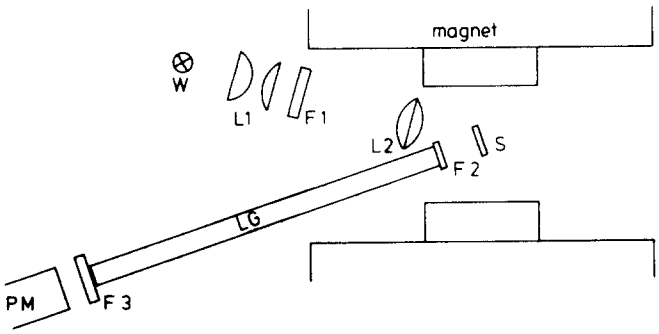

Fig. 2. Schematic representation of the apparatus used for fluorescence measurements. W, tungsten-iodine lamp; L1 and L2, lenses; S, sample cell; F, filters; F1, Balzers K2 and Schott BG12/4 mm; F2, Schott KV550; F3, Schott KV550 and Kodak Wratten 87C (for bacteria), or Schott KV550 + Schott RG665/3 mm + interference filter (for algae and chloroplasts); LG, 2-cm diameter lucite light guide; PM, photomultiplier tube (Dumont KM 2290 for bacteria, EMI 9658B for algae and chloroplasts). The measuring light intensity for bacteria was ~5 nE/cm^2.

grown and diluted with growth medium to give the desired absorbance. Chromatophores were prepared by sonication of cells in growth medium and resuspended after differential centrifugation in a buffer containing 50 mM morpholinopropane sulphonate and 50 mM KCl at pH 7. Chloroplasts were prepared as described elsewhere [14]. Fluorescence spectroscopy was carried out in a spectrophotometer mounted in a 9-inch Varian magnet (Fig. 2) [2]. For the experiments with bacterial cells and chromatophores an S1 type photomultiplier (Dumont KM2290) was used. This multiplier showed a small effect of the magnetic field on its sensitivity when placed near the magnet gap. With a 90-cm lucite light guide between sample cell and photomultiplier the apparent change in emission at $B = 0.6$ Tesla (6 kG) was smaller than the accuracy of the measurement ($5 \cdot 10^{-4}$ of the fluorescence), as checked with the fluorescing dye hexamethylindotricarbocyanine iodide. In bacteria appreciable fluorescence increases in a magnetic field occurred only after addition of dithionite. Fractional variations in emission in the unreduced controls, presumably in the state P^+, were less than 10^{-3}. This indicates that orientation effects are small. The temperature, which was varied by blowing cold nitrogen through a dewar vessel containing a sample cell of 2 mm thickness, was monitored with a Cu-constantan thermocouple extending into the cell.

Results

Fig. 3A shows a typical recording of the fluorescence F of whole cells of *Rps. sphaeroides* at room temperature in an external magnetic field, which was linearly increasing with time. In Fig. 3B part of this curve is redrawn as the relative change in fluorescence $\Delta F/F$ induced by the field. Fig. 3C exhibits the effect for chromatophores of *Rps. sphaeroides* at room temperature and at 175 K. It is seen that for both types of preparation a well-defined increase of fluorescence occurs with increasing value of B which saturates at $B \sim 0.15$ T. The value of B at which half the effect is attained ($B_{1/2}$) is about 25 mT. The curves appear complementary to the curves of the carotenoid triplet yield vs.

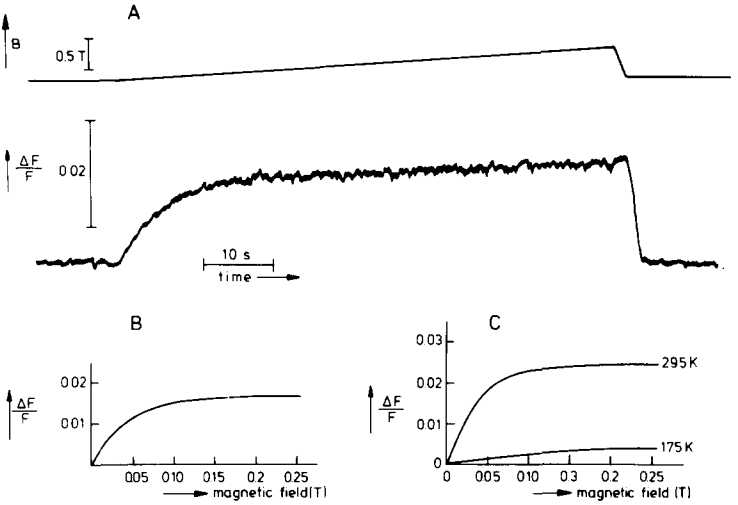

Fig. 3. (A) Upper trace: magnetic field as a function of time. The magnetic field was calibrated with an AEG NMR Gauss meter. Lower trace: resulting fluorescence change of *Rps. sphaeroides* cells, absorbance at 850 nm 0.2, reduced by adding solid sodium dithionite. (B) Same experiment, replotted as relative change of fluorescence $(F(B) - F(B = 0))/F(B = 0)$. (C) Relative fluorescence change of *Rps. sphaeroides* chromatophores, A 850 = 0.9 in a 5-mm cuvette as a function of magnetic field. Dithionite concentration 50 mM.

magnetic field previously measured [1,2]. The value of $B_{1/2}$ is approximately the same as found for the magnetodependence of the triplet yield *, which indicates that the phenomena are related.

The relative change in fluorescence at 0.5 T and $B_{1/2}$ is plotted as a function of temperature in Fig. 4 for whole cells of *Rps. sphaeroides*. It is seen that $\Delta F/F$, the fractional increase in fluorescence, shows a maximum at about 250 K, and that $B_{1/2}$ shows a minimum at about the same temperature. Chromatophores of *Rps. sphaeroides* showed a similar behaviour. The magnetic field effect for cells and chromatophores of the carotenoid-less mutant R-26 was similar to that for the wild-type, viz. $\Delta F/F$ (295 K) ~ 0.9%, $\Delta F/F$ (100 K) < 0.1%. For whole cells of *R. rubrum* at room temperature only a very low magnetic field effect was detected, $\Delta F/F \sim 0.2\%$, but for chromatophores an effect similar to that of *Rps. sphaeroides*, viz. $\Delta F/F \simeq 1.5\%$ and $B_{1/2} \sim$ 35 mT, which values decreased with lower temperatures.

In Fig. 5 the magnetic field effect for spinach chloroplasts and for *Cl. vulgaris* is displayed, measured at ~120 K. The curves are similar to those found for bacteria at higher temperatures. At temperatures above 0°C, the fluorescence of chloroplasts as well as of *Chlorella* showed a rather complex behaviour in a magnetic field with an initial increase and subsequent decrease with time on switching on the field. This complex pattern is probably caused in part by orientation of the pigment molecules by the magnetic field, as earlier observed and studied by Geacintov et al. [15], and was not further studied by us.

* As is apparent from Fig. 2A in Ref. 2, the value of $B_{1/2}$ for the triplet yield was 25 mT, and not 50 mT as inadvertently stated.

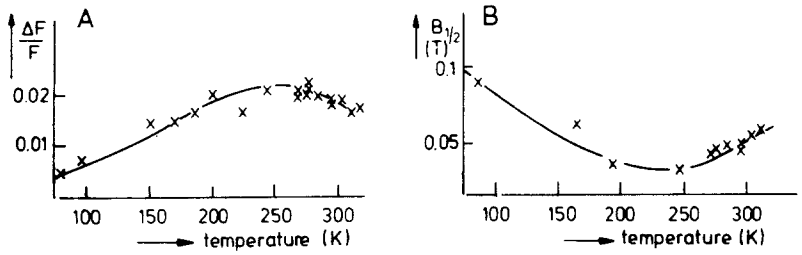

Fig. 4. (A) Relative fluorescence change induced by a magnetic field of 0.5 T in whole cells of *Rps. sphaeroides* with reduced acceptor X as a function of temperature. (B) Magnetic field value at which half the effect of 0.5 T is reached, as a function of temperature.

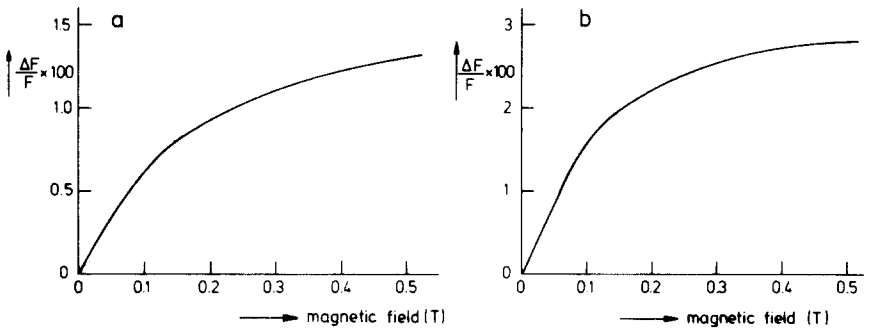

Fig. 5. Relative fluorescence change, measured at 685 nm, as a function of external magnetic field for: (a) *Cl. vulgaris;* absorbance 0.08 at 680 nm, temperature ~120 K, 10 μM 3-(3',4'-dichlorophenyl)-1,1-dimethylurea, and 10 mM hydroxylamine added; sample was frozen in the light. (b) spinach chloroplasts; chlorophyll concentration 33 μM, temperature ~120 K; dithionite added.

Discussion

The increase in fluorescence yield with a magnetic field of bacterial cells and chromatophores under reducing conditions may arise from a field-induced enhancement of the rate of the back reaction from the state P^+I^- to the excited singlet state P^*I as suggested in the Introduction. Starting with this hypothesis and making certain assumptions we can correlate quantitatively the fractional fluorescence change $\Delta F/F$ with the fractional change in triplet yield $\Delta P_T/P_T$ induced by the magnetic field. We find (see below) that $\Delta F/F$ is proportional to $\Delta P_T/P_T$ with a negative proportionality factor. This is in accordance with our finding that, at a given temperature, the fluorescence increase and the decrease in triplet yield saturate and reach the half-value at about the same field strengths.

In principle the above mechanism might be counteracted by another field-induced change in fluorescence yield. It has been shown that the probability for the conversion of the state P^+I^- to the triplet state P^TI is lower in a magnetic field [1,2]. This means that the concentration of reaction centers in the state $P\,I$ increases when applying a magnetic field and consequently the yield of fluorescence decreases. Hence it is important to assess the steady state concentration of reaction centers in the triplet state. At our measuring light

intensity ($\lesssim 5$ nE/cm^2) and sample absorbance (0.2), ~25 quanta are absorbed per s per reaction center. The reaction center triplet state is rapidly transferred to the reaction center carotenoid. During the lifetime of this carotenoid triplet, which is ~4 μs between 80 and 300 K, the trap is closed [10,16]. At lower temperatures the transfer to the carotenoid becomes inhibited and the lifetime of P^TI increases. Under our conditions, and assuming a 100% triplet yield, the fraction of reaction centers in the triplet state never exceeds 10^{-4}. Assuming 15% field induced depression of the triplet yield [2], the increase of the fraction of reaction centers in the ground state P I is smaller than $2 \cdot 10^{-5}$, so we can safely neglect its effect on the fluorescence.

At low temperature (1.4 K) yet another magnetic field effect on the fluorescence of photosynthetic bacteria has been reported [17]. This effect originates from field-induced mixing of populating and depopulating probabilities, which affects the concentration of the reaction center triplet state. At temperatures above say 20 K, however, this effect is abolished by rapid spin-lattice relaxation between the triplet sublevels.

As mentioned in the previous section, in some instances orientational effects can be important. For chromatophores and whole bacterial cells however, these effects are negligible at room temperature, as demonstrated by the absence of a magnetic field effect in non-reduced samples. We assume that in all preparations at low temperatures, no field-induced orientation will occur.

We will now derive the relationship between triplet yield and fluorescence. To this end we first calculate P_F, the total probability that an excitation is emitted as fluorescence [11]:

$$P_F = (1 - T)p_f + Tp_f^t + (1 - T)p_h P'_F + Tp_h^t P''_F$$

P'_F is the probability for fluorescence after one excitation transfer step, P''_F is the probability for fluorescence after returning from the reaction center, p_f and p_f^t are the probabilities for immediate emission of fluorescence for an antenna bacteriochlorophyll and the reaction center, respectively, p_h and p_h^t are the probabilities for energy transfer, T is the fractional concentration of reaction centers. If we assume $P_F = P'_F = P''_F$, we get

$$P_F = \frac{(1 - T)p_f + Tp_f^t}{1 - (1 - T)p_h - Tp_h^t} \tag{1}$$

and its analogue for the total triplet yield

$$P_T = \frac{(1 - T)p_{isc} + Tp_t^t}{1 - (1 - T)p_h - Tp_h^t} \tag{2}$$

where p_{isc} is the probability for intersystem crossing in an antenna bacteriochlorophyll and p_t^t the probability that a reaction center triplet is formed from P^*I. Assuming that the rate constants for deexcitation and energy transfer are equal for antenna and reaction center chlorophylls, we can express P_F and P_T in terms of these rate constants, the rate constants for charge separation and recombination, and ϕ_s, the probability that P^+I^- will recombine to the excited singlet state P^*I:

$$P_F = \frac{k_f(1 - AT)}{k_1 + k_h AT} \tag{3}$$

$$P_T = \frac{k_{isc}(1 - AT) + kAT(1 - (1 + \alpha)\phi_s)/(1 - \phi_s)}{k_1 + k_h AT} \tag{4}$$

with $A = k_c(1 - \phi_s)/(k + k_c(1 - \phi_s))$ and $\alpha = k_g/k_s$. The rate constants are defined in Fig. 1. $k_1 = k_f + k_{ic} + k_{isc}$ is the rate of deexcitation of an antenna bacteriochlorophyll molecule other than via energy transfer. $k = k_1 + k_h$ is the total deexcitation rate. We assume that the rate constants are not altered by a magnetic field. The only parameter that is influenced by the field is ϕ_s. Elimination of ϕ_s yields

$$P_T = -BP_F + C \tag{5}$$

with

$$B = 1 + [k_{ic} + \alpha(k_1 + (k_1 + k_h T)k_c/k)]/k_f \tag{6}$$

and

$$C = 1 + \alpha(1 + (1 - T)k_c/k) \tag{7}$$

From Eqns. 5, 6 and 7 the relationship between the fractional yields of fluorescence and total triplet yield is given by

$$\Delta F/F = \Delta P_F/P_F = -[P_T/(C - P_T)][\Delta P_T/P_T] \tag{8}$$

P_F and P_T are the fluorescence yield and triplet yield in zero magnetic field. If we assume $k_g = 0$, the triplet yield P_T at 80 K will be close to 1. We now consider changes caused by a saturating magnetic field. At room temperature P_T was estimated to be ~0.3 [11], so that $P_T/(C - P_T) \simeq 0.3/(1 - 0.3) \simeq 0.43$. With $\Delta P_T/P_T \simeq -0.05$ (Rademaker, H., unpublished), the right hand side of Eqn. 8 is equal to $0.43 \cdot 0.05 = 0.022$, in good agreement with our measured value $\Delta P_F/P_F \simeq 0.018$. Since we have assumed $k_g = 0$, in whole cells of *Rps. sphaeroides* only a small part of the radical pairs apparently combines to the ground state. In chromatophores we measured $\Delta P_T/P_T \simeq -0.12$ [2]. If we assume again $k_g = 0$ and $P_T = 0.3$, the right hand side of Eqn. 8 is equal to 0.05, a factor 2 different from our measured value $\Delta P_F/P_F = 0.024$. Our assumption $k_g = 0$ may not be correct for this case. If $k_g > 0$, a higher value of C is obtained resulting in a lower calculated value of $\Delta F/F$. Alternatively, P_T may have a lower value, e.g. caused by a less efficient energy transfer between the antenna pigments and the reaction center.

If we assume that the temperature dependence of $\Delta P_T/P_T$ is similar to that found in reaction centers [1], we can explain the rise in $\Delta F/F$ in the temperature region of 80–250 K by the rise in $|\Delta P_T|/P_T$, and the decrease at higher temperatures by the decrease of $P_T/(C - P_T)$, due to the decrease of P_T [11]. The curve of $B_{1/2}$ vs. T can be qualitatively understood with the aid of the model calculations of Werner et al. [18]. These authors predict a decrease in $B_{1/2}$ with decreasing k_s, owing to the corresponding sharpening of the radical pair singlet level (the fuzzier the levels are, the higher a magnetic field one needs to pull $T_{\pm 1}$ out of the region where these levels can still mix with the singlet level). The minimum in $B_{1/2}$ can be explained by an increase in k_t with decreasing T, which below 240 K takes over the effect of decreasing k_s.

Because of lack of data on P_T, it is as yet not possible to apply analogous

calculations for the magnetic field effect of the Photosystem II fluorescence of chloroplasts and *Chlorella*. The similarity between the low field experimental results obtained from these preparations with the results found for the bacterial system suggests that the radical pair mechanism is operative for Photosystem II also. This indicates that an intermediate acceptor is present between the primary donor of Photosystem II, *P*-680, and the primary acceptor, Q. Recent results obtained with optical difference spectroscopy suggests that such an intermediate might be pheophytin [19].

Note added in proof (Received January 31st, 1979)

We have learned that Voznyak et al. [20] have carried out similar experiments in purple bacteria and arrived in a qualitative way at similar conclusions as we did.

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