Biochimica et Biophysica Acta, 592 (1980) 235—239

BBA 47906

MAGNETIC FIELD AFFECTS THE FLUORESCENCE YIELD IN REACTION CENTER PREPARATIONS FROM *RHODOPSEUDOMONAS SPHAEROIDES* R-26

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(Received December 14th, 1979)
(Revised manuscript received March 10th, 1980)

Key words: Bacteriochlorophyll; Fluorescence yield; Magnetic field effect; Photosynthetic bacterium; Reaction center; (Rhodopseudomonas sphaeroides)

Summary

Purified photochemical reaction centers from *Rhodopseudomonas sphaeroides* R-26 were reduced with $Na_2S_2O_4$ so as to block their photochemical electron-transfer reactions. The magnetic field induced an increase in the emission yield. Our results support the hypothesis that under these conditions, charge recombination in the singlet radical pair composed of the oxidized primary donor and reduced primary acceptor predominantly generates the excited singlet state of the reaction center bacteriochlorophyll.

The maximum relative fluorescence change and the value of the magnetic field at which half-saturation of the effect is achieved ($B_{1/2}$) at room temperature are 5.5% and 75 G, respectively. For the whole cells of *Rps. sphaeroides* R-26 these parameters are 1.2% and 120 G.

The relative fluorescence change at 600 G, $\Delta F/F(600)$, and $B_{1/2}$ are studied as functions of temperature. The temperature dependencies of $\Delta F/F(600)$ for reaction centers and whole cells of *Rps. sphaeroides* R-26 are qualitatively the same, with the maximum effect (8% for reaction centers) occurring at 230 K. However, the $B_{1/2}$ curves for the two preparations are different.

Introduction

The transformation of light energy into chemical energy in the photosynthetic apparatus of bacteria originates from photo-induced electron-transfer reactions in a membrane-bound complex of pigments and proteins, the reaction center, to which the absorbed light energy is channeled. The reaction

centers contain four molecules of bacteriochlorophyll and two molecules of bacteriopheophytin [1,2]. The electron flow following the excitation of ^1P, a bacteriochlorophyll dimer (*P*-870), is started by the formation of a radical ion pair [3,4] (called state P_F) in less than 10 ps:

$$(^1P^{*1}I)^1X^- \rightarrow {}^1(^2P^{+2}I^-)^1X^-$$

The electron acceptor, I, is generally assumed to be bacteriopheophytin [4—6]. Under normal conditions the electron is transferred within 100—250 ps [6,7] from I^- to the second acceptor, X, an iron-quinone complex [1]. If the photochemical electron-transfer reaction is blocked by chemical reduction of the iron-quinone complex, the life-time of the radical pair in isolated reaction centers increases to about 10 ns [8] at room temperature. This time reflects the rate of electron back transfer and it is sufficient for singlet-triplet interconversion in the radical pair to take place.

In case the pair is in the singlet electron spin state, either the singlet ground state ($^1P^1I$) or the singlet excited state ($^1P^{*1}I$) is populated after recombination. Indication of an efficient back reaction to $^1P^{*1}I$ has been obtained by the study of the fluorescence yield as a function of temperature in bacterial cells at low redox potentials [9,10].The various possible reaction pathways are schematically represented in Refs. 10 and 11. From $^3(^2P^{+2}I^-)$ the charges will recombine to form the reaction center triplet state, ^3P.

Recently, it has been found that a static magnetic field decreases the quantum yield of the photo-induced triplet state and increases the emission yield in photosynthetic bacteria in which X is pre-reduced [11—14].

The relative magnetic field-induced changes of the fluorescence yield of chemically reduced chromatophores and whole cells of different photosynthetic bacteria do not exceed 1—3% [11,14]. It is expected that larger effects will be characteristic of reaction center preparations devoid of the light-harvesting pigments the fluorescence of which is insensitive to the magnetic field.

The fluorescence quantum yield of intact reaction centers has been determined to be 0.03—0.04% [15]. If the acceptor, X, is chemically reduced the fluorescence quantum yield increases up to 0.12—0.15% [15,16], presumably due to delayed fluorescence as a result of the recombination, $^1(^2P^{+2}I^-) \rightarrow {}^1P^{*1}I$. In this case, the magnetic field can affect only delayed fluorescence of *P*-870. Here we study the magnetic field effect on the delayed fluorescence in reaction center preparations from *Rhodopseudomonas sphaeroides* R-26 over the temperature range from +60 to —160°C.

Materials and Methods

Reaction center preparations of *Rps. sphaeroides* R-26 were isolated as described elsewhere [17]. After dialysis they were transferred to a medium containing 50 mM Tris-HCl (pH 8.5). Experimental conditions are described in Ref. 11. The signal of fluorescence from a cooled S-1 type photomultiplier was measured by a digital voltmeter operating on line with a desk-top calculator. A linear 0—600 G field sweep was obtained by means of a pair of coils in a Helmholtz configuration. The sweep speed was 200 G/s. The whole system worked as a synchronous differential signal averager. The magnetic field was

calibrated by a Hall probe gaussmeter. The maximal magnetic field effect on the photomultiplier current and the exciting light source was less than the accuracy of measurement (0.01%).

Fluorescence measurements were carried out in a 2 mm thick flat cell. The excitation light source was a high-pressure xenon arc filtered by an 8 cm heat filter and a glass filter, transmitting the 450—610 nm band. The incident power was about 10 mW/cm^2. Fluorescence was detected via an interference filter with λ_{max} = 910 nm. The temperature, which was varied by blowing cool N_2 through a Dewar tube containing the sample, was monitored with a copper-constantan thermocouple and a second digital voltmeter. The heat inactivation of samples was carried out by subjecting them to a temperature of 55°C for 10 min. After this treatment the photo-induced changes at 870 nm were zero.

Results

Fig. 1 shows a typical measurement of relative fluorescence changes with respect to the magnetic field taken at different temperatures. The maximum relative fluorescence change and the value of the magnetic field at which half-saturation of the effect is achieved ($B_{1/2}$) at room temperature are 5.5% and 75 G, respectively. For whole cells of *Rps. sphaeroides* R-26 these parameters are 1.2% and 120 G, respectively [11]. In reaction center preparations with an oxidised acceptor, X, or in heat-inactivated samples the magnetic field effect is absent.

The relative fluorescence changes at 600 G and $B_{1/2}$ are plotted in Figs. 2 and 3 as functions of temperature. The temperature dependencies of $\Delta F/F(600)$ for reaction centers and whole cells of *Rps. sphaeroides* R-26 are qualitatively the same, with the maximum change being 8% for reaction centers occurring at 230 K (Fig. 2). Alternatively, the $B_{1/2}$ curves for the two preparations demonstrate different behavior (Fig. 3).

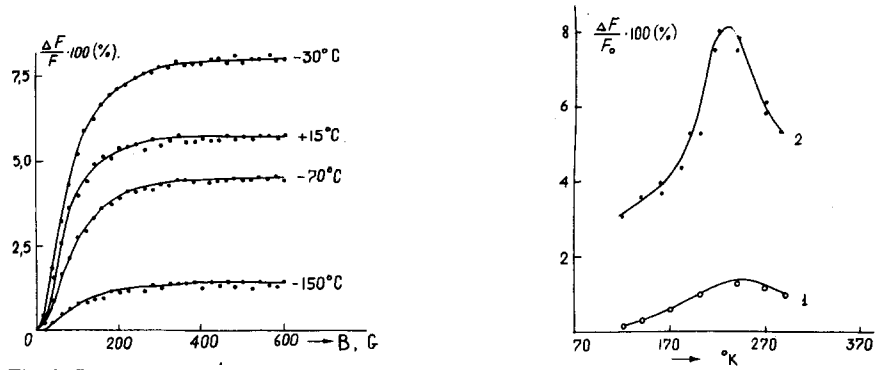

Fig. 1. Relative fluorescence changes, $\Delta F/F(B) = (F(B) - F(0))/F(B)$, of the chemically reduced reaction center preparation from *Rps. sphaeroides* R-26 measured at 910 nm, as a function of the external magnetic field, at several temperatures. The samples contained 80% glycerol and 2 mM sodium dithionite (E_m = —250 mV). The number of averaged scans is 100.

Fig. 2. Maximum relative fluorescence change, $\Delta F/F(B)$, induced by a magnetic field of 600 G in the whole cells of *Rps. sphaeroides* R-26 (1) and isolated reaction centers (2), with reduced acceptor (X), as a function of temperature. The experimental conditions are the same as in Fig. 1.

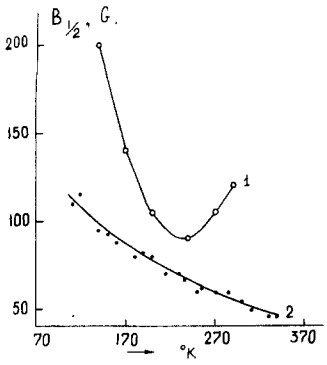

Fig. 3. Magnetic field value ($B_{1/2}$) at which half of the maximum effect is reached as a function of temperature: (1) whole cells; (2) isolated reaction centers. The experimental conditions are the same as in Fig. 1.

Discussion

The experimental results are indicative of the back electron transfer reaction:

$$^1(^2P^{+2}I^-) \rightarrow (^1P^{*1}I)$$

In an external magnetic field the triplet level of the radical pair splits into three sublevels, T_{+1}, T_0, T_{-1}, corresponding to the values of the spin quantum number m_S. The sublevels are separated by the Zeeman energy, $g\beta B$. If the exchange interaction in the radical pair $^{1,3}(^2P^{+2}I^-)$ is absent, in a strong magnetic field only the T_0 level is sufficiently close to the singlet level for the interconversion, $^1(^2P^{+2}I^-) \leftrightharpoons {}^3(^2P^{+2}I^-)$, to take place [18]. This means that, while in the absence of a magnetic field all three sublevels of the $^3(^2P^{+2}I^-)$ state are effective, the application of a field reduces the number of functioning levels to one. It decreases the fraction of the $^3(^2P^{+2}I^-)$ state and increases the fraction of the $^1(^2P^{+2}I^-)$ state. This in turn leads to an increase in the emission yield.

Both the value of $B_{1/2}$ and its temperature dependence differ for the whole cells and the reaction center preparations of *Rps. sphaeroides* R-26. Earlier it was pointed out [19] that the $B_{1/2}$ value is determined predominantly by the values of the rate constants for recombination of the $^1(^2P^{+2}I^-)$ state to the fluorescent state (K_r) and the same process for $^3(^2P^{+2}I^-)$ leading to the triplet state of *P*-870 (K_T). According to Ref. 19 changes of the K_r and K_T values are paralleled by the corresponding changes of the $B_{1/2}$ values.

Recombination of the singlet radical pair is thermally activated (E_a = 0.12 eV for whole cells) [10]. This means that the values of K_r, together with the $B_{1/2}$ values, will decrease when the temperature is decreased. This case is experimentally observed in the 230—295 K temperature range for the whole cells (Fig. 3) [14]. The lack of such temperature dependence for the reaction centers leads us to an assumption that the energy gap between the excited and charge-transfer states is smaller in the reaction centers. The $B_{1/2}$ increase for the whole cells and reaction center preparations may be caused by an increase in the K_T value. The change of K_T could be related to a conformational change of the reaction center with decreasing temperature [20].

Our conclusion concerning this possibility is further supported by the data on the gradual change of the curve of field dependence with temperature observed for the whole cells and chromatophores of *Rps. sphaeroides* [21]. The results presented in Ref. 21 indicate the decrease in distance between the partners in a radical pair and an increase in the exchange interaction in the same temperature range.

For the explanation of the complex temperature dependence of the maximal magnetic field effect (Fig. 2) in the frame-work or Ref. 19 one should assume corresponding temperature dependencies of the rate constants, K_r, K_T and K_g (the latter stands for the process of a direct recombination of $^1(^2P^{+2}I^-)$ to the ground state). However, theoretical and experimental information on such temperature dependencies is lacking. Attempts to rationalize the observed temperature dependence of the magntic field effects are so far speculative.

The large magnetic field effect observed in the reaction center preparations confirms the assumption that the field effects observed for chromatophores and whole cells arise from the same mechanism, i.e., the magnetic field-induced changes of the fraction of fluorescence resulting from recombination of the radical pair.

Acknowledgements

We would like to thank I.I. Proskuryakov for discussions, A.P. Kazantsev for technical assistance and Yu.E. Erokhin for his gift of the reaction center preparation.

References

1 Parson, W.W. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 416, 105—149
2 Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265—278
3 Thurnauer, M.C., Katz, J.J. and Norris, J.R. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3270—3274
4 Dutton, P.L., Kaufmann, K.J., Chance, B. and Rentzepis, P.M. (1975) FEBS Lett. 60, 275—280
5 Fajer, J., Brune, D.C., Davis, M.S., Forman, A. and Spaulding, L.D. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4956—4960
6 Rockley, M.G., Windsor, M.W., Cogdell, R.J. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2251—2255
7 Kaufmann, K.J., Dutton, P.L., Netzel, T.L., Leigh, J.S. and Rentzepis, P.M. (1975) Science 188, 1301—1304
8 Cogdell, R.J., Monger, T.G. and Parson, W.W. (1975) Biochim. Biophys. Acta 408, 189—199
9 Holmes, N.G., van Grondelle, R., Hoff, A.J. and Duysens, L.N.M. (1976) FEBS Lett. 70, 185—190
10 Van Grondelle, R., Holmes, N.G., Rademaker, H. and Duysens, L.N.M. (1978) Biochim. Biophys. Acta 503, 10—25
11 Voznyak, V.M., Elfimov, E.I. and Proskuryakov, I.I. (1978) Doklady Acad. Nauk U.S.S.R. 242, 1200—1203
12 Blankenship, R.E., Schaafsma, T.J. and Parson, W.W. (1977) Biochim. Biophys. Acta 461, 297—305
13 Hoff, A.J., Rademaker, H., van Grondelle, R. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 460, 547—554
14 Rademaker, H., Hoff, A.J. and Duysens, L.N.M. (1979) Biochim. Biophys. Acta 546, 248—255
15 Zankel, K.L., Reed, D.W. and Clayton, R.K. (1968) Proc. Natl. Acad. Sci. U.S.A. 61, 1243—1249
16 Slooten, L. (1972) Biochim. Biophys. Acta 256, 452—466
17 Okamura, M.L., Steiner, L.A. and Feher, G. (1974) Biochemistry 13, 1394—1402
18 Buchachenko, A.L. (1974) Chemical Polarization of Electrons and Nuclei, Nauka, Moscow
19 Werner, H.J., Schulten, K. and Weller, A. (1978) Biochim. Biophys. Acta 502, 255—268
20 Hales, B.J. Biophys. J. (1976) 16, 471—480
21 Elfimov, E.I., Voznyak, V.M. and Kazantzev, A.P. (1980) Biofizika 25, N 3, in the press