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MAGNETIC FIELD-INDUCED FLUORESCENCE CHANGES IN CHLOROPHYLL-PROTEINS ENRICHED WITH *P*-700

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Summary

Fluorescence yield dependence on external magnetic field (0—600 G) was measured for chlorophyll-protein complexes enriched with Photosystem I. Maximal relative changes of fluorescence yield at room temperature (1.0—2.5%) were dependent on the chlorphyll *a*:*P*-700 ratio. Magnetic field-induced changes were observed only in the presence of dithionite. At low temperatures (down to —160°C) the magnetic field-induced effect decreased. The effect is obviously connected with the functions of reaction centers in Photosystem I. An explanation of the effect is proposed based on the hypothesis of radical pairs recombination within the reaction center. For the radical pair ($P\text{-}700^{+\cdot}$ $A^{-\cdot}$), an intermediate acceptor, $A^{-\cdot}$, with a *g*-value approximately equal to that of $P\text{-}700^{+\cdot}$ is proposed.

Magnetic field-induced fluorescence changes at room temperature have been observed in photosynthetic bacteria as well as in algae and spinach chloroplasts [1, 2]. Since the changes in fluorescence yield of bacterial preparations correlated with a decrease of the yield of triplet states in reaction centers [3, 4], the effect was associated with the primary reaction of charge separation and explained by a radical pair recombination hypothesis [1—4]. In the case of plant photosynthesis the observed magnetic field-induced effects have been ascribed to the evolution of a radical pair appearing in Photosystem II. Here we report magnetic field-induced fluorescence changes in Photosystem I.

The experiments were made on *P*-700-enriched complexes isolated from

pea chloroplasts according to Thornber [5]. The *P*-700/chlorophyll *a* ratio was 1/(30—50). The complexes contained only the polypeptides characteristic of Photosystem I [5]. At room temperature the complexes fluoresced with a maximum at 684—685 nm. The redox states were poised by addition of sodium dithionite and neutral red and were monitored by light-induced absorbance changes at 430 and 700 nm according to Ref. 6.

Addition of reductant and mediator caused an increase of relative fluorescence yield of 60—70% in accordance with Ref. 7. Absorbance at 676 nm did not exceed 0.5 for 1-cm cuvette. For fluorescence measurements a 1-cm cuvette placed in the electromagnet gap was illuminated by broad-band (400—600 nm) light from a 75 W halogen incandescent lamp. The intensity of the exciting beam was 10^3—10^4 ergs·cm^{-2}·s^{-1}. Fluorescence intensity was monitored with a photomultiplier with a cut-off filter, $\lambda > 680$ nm. The magnetic field strength was swept by a ramp generator. The photomultiplier signals were averaged over 100 sweeps with an on-line 15-BCM-5 microcomputer. The details of the apparatus will be published elsewhere. During each set of measurements the temperature of the sample was kept constant and monitored with a thermocouple.

Changes of fluorescence yield were characterized by

$$\Delta F(H)/F = (F(H) - F(0))/F(0)$$

$F(0)$ and $F(H)$ being fluorescence intensities measured at zero magnetic field and at magnetic field H. Fig. 1 shows typical magnetic field-induced effects in chlorophyll-proteins enriched with *P*-700. These effects were observed only in the presence of dithionite or dithionite and neutral red. No magnetic field-induced effects were seen in light-harvesting chlorophyll-protein complexes from pea chloroplasts or in *P*-700-enriched complexes with thermoinactivated reaction centers. It follows that magnetic field-induced fluorescence changes depend on functional activity and processes in the reaction center of Photosystem I (PS I).

Fig. 2 shows magnetic field-induced effects at various temperatures. The decrease of $\Delta F(H)/F$ and the changes in the shape of the curve at low temper-

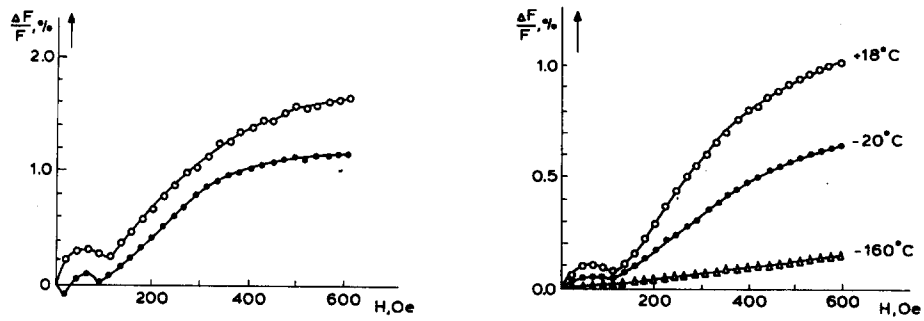

Fig. 1. Magnetic field-induced changes of relative fluorescence yield in chlorophyll-protein complexes enriched with *P*-700. 20 mM Tris-HCl buffer, pH 8. Additions: $Na_2S_2O_4$, 2 mg/ml; neutral red, $2 \cdot 10^{-5}$ M. Room temperature. The two curves correspond to different isolations of chlorophyll-protein complexes.

Fig. 2. Magnetic field-induced changes of fluorescence yield at various temperatures. The samples contained 70% (v/v) glycerol. The other conditions were as in Fig. 1.

atures were completely reversible. In the primary reaction of PS I in higher plant photosynthesis, the electron donor *P*-700 is oxidized and electrons are transferred via intermediate and primary acceptors:

$$P\text{-}700 \xrightarrow{e^-} A_1 \xrightarrow{e^-} A_2 \xrightarrow{e^-} P\text{-}430\ (A, B)$$

P-700 being a chlorophyll *a* dimer [8], acceptors *P*-430 (A, B) and A_2 being iron-sulfur proteins [9]. The nature of A_1 is not clear so far; it was shown to have certain properties of an organic molecule [10—12], perhaps a chlorophyll *a* dimer [13].

Magnetic field-induced effects in photosynthetic bacteria and algae were explained via a hypothesis of radical pair recombination in reaction centers [1—4]. Upon photooxidation of the electron donor, P, (within 10 ps [10]), a radical pair is generated in its singlet state $^1(P^{+\cdot}\ A^{-\cdot})$. The lifetime of the radical pair depends on the redox state of the secondary acceptors, and at low redox potentials it may be long enough for intersystem conversion to a triplet state $^3(P^{+\cdot}\ A^{-\cdot})$. Recombination processes that take place in triplet radical pairs lead to triplet (P^TA) formation, whilst those in singlet radical pairs regenerate the ground (PA) or the singlet excited (P^*A) states of reaction centers. In the latter case there is a non-zero probabiblity for recombination fluorescence:

$$^1(P^{+\cdot}\ A^{-\cdot}) \rightarrow (PA) + h\nu_{fl}$$

Since the probability of intersystem conversion $^1(P^{+\cdot}\ A^{-\cdot}) \rightleftharpoons {}^3(P^{+\cdot}\ A^{-\cdot})$ depends on the intensity of external magnetic field [14], the triplet (P^TA) yield and fluorescence yield are also field-dependent. Two mechanisms were proposed for the dependence [14]:

(1) Δg: the mechanism that dominates in intense magnetic fields for radical pairs with the partners differing significantly in *g* value ($\Delta g = |g_1 - g_2| > 10^{-3}$). In this case, a magnetic field speeds up the singlet-triplet conversion and decreases the fluorescence yield.

(2) The hyperfine coupling mechanism is important in moderate magnetic fields for radical pairs with $\Delta g < 10^{-3}$. If the mechanism is operative, all three degenerate sublevels of the triplet radical pairs are populated in zero magnetic field. However, an external magnetic field of sufficient strength lifts the degeneracy and the population of only the To sublevel occurs, thus decreasing the probability of S-T conversion in radical pairs. In this case a magnetic field increases the fluorescence yield. In the framework of this hypothesis the fluorescence increase shown in Figs. 1 and 2 implies the hyperfine coupling mechanism [14] and leads us to the conclusion that the *g* value for $A^{-\cdot}$ is close to that of $P\text{-}700^{+\cdot}$ (i.e. 2.0026). A species with a *g* value near 2.00 is usually an organic molecule. In our experiments the addition of the dithionite and neutral red at the light intensities used for exitation led to accumulation of $P\text{-}430(A,B)^-$ in illuminated samples. We cannot exclude accumulation of A_2^- as well. In the latter case a *g* value near 2.0026 proposedly is a characteristic of the intermediary acceptor A_1. In any case, we conclude that in PS I between *P*-700 and *P*-430 there exists an intermediate acceptor with a *g* value near 2.0026. This is in accord with independent findings of other

investigators [10—13]. If, in fact, a chlorophyll-type molecule is identical with A, then the Δg value for $P\text{-}700^{+\cdot}$ and $A_1^{-\cdot}$ is about 0.0005 (the g factor of $Chl^{-\cdot}$ 2.0031 [15, 16]). A detailed analysis of the shape of the $\Delta F(H)/F$ curve (Figs. 1 and 2) leads to an assumption of the existence of two magnetic field-sensitive processes. This assumption is further supported by the preliminary experimental data on the dependence of the shape of the curve of the magnetic field effect on the detection wavelength (Fig. 3). If the detection wavelength is shifted to 725 nm, the negative fluorescence changes appear (Fig. 3B). The experimental curves (Figs. 1 and 3A) can be simulated by a sum of a positive monotonic component and a non-monotonic component, the latter demonstrating negative magnetic field effect in weak fields [17]. The negative branch of the curve can be explained by an exchange interaction with a characteristic value of 30—50 G.

If our reasoning is correct, then it may be stated that the primary processes of the plant photosynthesis are very much like those of the bacterial photosynthesis. In the case of photosynthetic bacteria a spin-polarized reac-

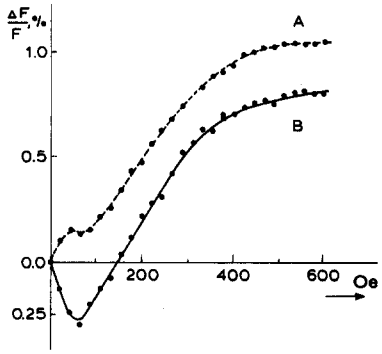

Fig. 3. Magnetic field-induced changes of fluorescence yield at different wavelengths of detection. A, $\lambda > 660$ nm; B, $\lambda = 725 \pm 5$ nm. The other conditions were as in Fig. 1.

tion center triplet is readily observed by ESR in chemically reduced samples [18, 19]. The lack of the data on the observations of the triplet states of the PS I reaction centers is puzzling. However, this discrepancy seems to have been resolved in recent work [20], in which the triplet states related to the PS I reaction centers were reported. The conditions of these measurements were similar to ours, with the exception of the temperature, which was in the liquid helium range. The polarization of the triplets confirms strongly their evolution in the radical-pair recombination process. This serves as an independent proof of the above proposed mechanism of the magnetic field effects.

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References

1 Voznyak, V.M., Elfimov, E.I. and Proskuryakov, I.I. (1978) Dokl. Acad. Nauk. U.S.S.R. 242, 1200—1203

2 Rademaker, H., Hoff, A.J. and Duysens, L.N.M. (1979) Biochim. Biophys. Acta 546, 248—255
3 Blankenship, R.E., Schaafsma, T.J. and Parson, W.W. (1977) Biochim. Biophys. Acta 462 297—305
4 Hoff, A.J., Rademaker, H., van Grondelle, R. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 460, 547—554
5 Shiozawa, J.A., Albert, R.S. and Thornber, J.R. (1974) Arch. Biochem. Biophys. 165, 388—396
6 Ke, B. (1973) Biochim. Biophys. Acta 301, 1—33
7 Telfer, A., Barber, J., Heathcote, P. and Evans, M.C.W. (1978) Biochim. Biophys. Acta 504, 153—164
8 Katz, J.J. and Norris, J.R. (1978) Annu. Rev. Biophys. Bioeng. 7, 393
9 Malkin, R. and Bearden, A.J. (1978) Biochim. Biophys. Acta 505, 147—181
10 Fenton, J.M., Pellin, M.J., Govindjee and Kaufmann, K.J. (1979) FEBS Lett. 100, 1—4
11 Dismukes, G.S., McGuire, A., Blankenship, R. and Sauer, K. (1978) Biophys. J. 21, 239—256
12 McIntosh, A.R., Manikowski, M., Wong, S.K., Taylor, C.P.S. and Bolton, J.R. (1979) Biochem. Biophys. Research Commun. 87, 607—612
13 Shuvalov, V.A., Dolan, E. and Ke, B. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 770—773
14 Sagdeev, R.Z., Salihov, K.M. and Molin, Y.N. (1977) Usp. Chimii (Russan edn.) 46, 569—601
15 Kim, V.A. (1977) Dissertation, Moscow
16 Fujita, I., Davis, M.S. and Fajer, J. (1978) J. Am. Chem. Soc. 100, 6280—6282
17 Voznyak, V.M., Ganago, I.B., Moskalenko, A.A. and Elfimov, E.I. (1979) Studia Biophysica 77,
18 13—20
19 Uphaus, R.A., Norris, J.R. and Katz, J.J. (1974) Biochem. Biophys. Res. Commun. 61, 1057—1063
20 Frank, H.A., McLean, M.B. and Sauer, K. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5124—5128