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Magnetic-field effects on primary reactions in Photosystem I

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Magnetic-field effects on primary reactions in Photosystem I have been studied by measuring flash-induced absorption changes at 820 nm in SDS-CP1 particles in magnetic fields up to 1000 G. The amplitude of a transient decay phase with $t_{1/2} \approx 6$ μs, which is attributed to the triplet state of P-700 formed by charge recombination in the primary radical pair ($P\text{-}700^+\text{–}A_0^-$), shows a clear increase with increasing field (maximum effect at around 60 G), followed by a decrease to a level well below the control without field. The magnetic-field dependence of the triplet yield is discussed with regard to magnetic interactions between $P\text{-}700^+$ and A_0^-: an exchange interaction $|2J|$ of approx. 60 G is deduced (in accordance with a previous estimation of 60–100 G: Voznyak, V.M., Ganago, I.B., Moskalenko, A.A. and Elfimov, E.I. (1980) Biochim. Biophys. Acta 592, 364–368).

Introduction

In photosynthetic reaction centers, the conversion of light energy into chemical energy is initiated, after excitation of the primary donor P to its first excited singlet state, by a charge separation: $P^*\text{–}A_0 \rightarrow P^+\text{–}A_0^-$, where A_0 is the primary electron acceptor. Under physiological conditions, the electron on A_0^- is further transferred to a secondary acceptor A_1 in a subnanosecond reaction. However, the latter step can be blocked by chemical reduction or extraction of A_1. The radical pair ($P^+\text{–}A_0^-$) then decays by recombination with a much longer halftime (for example, about 10 ns in purple bacteria) and some triplet state ^3P of the primary donor is formed through this recombination reaction.

The effects of external magnetic fields on these primary reactions and especially on the yield of formation of ^3P have been extensively studied in reaction centers of purple bacteria (for reviews on experimental results and their interpretation, see Refs. 1–3), whereas only a few studies on such effects have been reported for reaction centers of higher plants (Ref. 4; see also references in Ref. 1), and have been interpreted in the framework of the radical pair mechanism which is described briefly in the following (Fig. 1).

When electron transfer to A_1 is blocked, the lifetime of the radical pair ($P^+\text{–}A_0^-$) is sufficiently large so that the spin multiplicity of the pair can evolve from singlet to triplet, and some ^3P triplet state is formed by recombination from the triplet state of the radical pair. In relatively weak magnetic fields ($0 \leqslant B \leqslant 1$ kG), hyperfine coupling is the operative mechanism for these transitions which can mix the singlet state with the three

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Abbreviations: ΔA, absorption change; PS I, Photosystem I.

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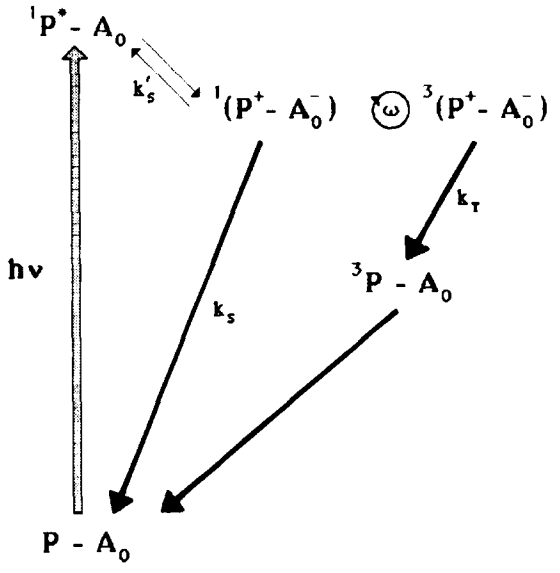

Fig. 1. Reaction scheme for the primary radical pair of photosynthetic reactants when electron transfer to further acceptors is blocked.

triplet sublevels in zero field, whereas only S-T_0 mixing is possible for higher fields, due to the fact that the energies of the T_{+1} and T_{-1} states are separated from the singlet state energy by the Zeeman interaction. Hence, in the presence of a magnetic field, the yield of ^3P formation will be decreased and the lifetime of the primary radical pair (P^+–A_0^-) can be modified due to different decay rate constants k_S and k_T of the singlet and triplet states of the pair. This picture may be complicated by the fact that lifetime broadening (due to k_S and k_T) as well as magnetic interactions (exchange and dipolar) between P^+ and A_0^- cannot be neglected. The magnetic interactions hinder the singlet-triplet mixing at zero field (or high fields), as they remove the degeneracy between the singlet and triplet states (or S and T_0 states). In principle, these parameters could be extracted from the magnetic field dependence of the dynamics of the pair and the yield of ^3P formation, but this has not been accomplished yet unambiguously even for reaction centers of purple bacteria which have been thoroughly studied.

We present here data concerning essentially the magnetic-field dependence of formation of the P-700 triplet state in higher plants (P-700 being the primary electron donor of Photosystem I, probably a dimer of chlorophyll *a*) which has been formed by charge recombination between P-700$^+$ and A_0^-, where the primary acceptor A_0 of Photosystem I is probably a specialized chlorophyll *a* molecule.

Materials and Methods

CP1 particles containing A_0 as the only functional electron acceptor were prepared from spinach [5] at approx. 0.3 mg chlorophyll/ml, except that 0.1% SDS was left in the preparation in order to avoid aggregation of the particles.

Flash-induced absorption changes at 820 nm were measured essentially as in Ref. 6 with the following modifications: excitation pulses at 532 nm were of 30 ps duration (full width) at a repetition rate of 1 Hz (Quantel YG 402). The measuring light was detected by a rapid silicon photodiode (Lasermetrics 3117). For measurements with electrical bandwidths of 500 Hz–500 MHz or DC–1 MHz AC, respectively, the signals were first amplified (10-20-1C amplifier from Nucleotides or plug-in amplifier 7A22 from Tektronix) and then recorded by a Tektronix 7912 digitizer interfaced with a signal averager (Didac, Intertechnique) or by a Biomation 2805 digitizer coupled to a Tracor 1710. The rectangular sample cuvette (optical path: 10 mm in the direction of the measuring beam and 2 mm in the direction of the excitation beam) was placed in the center of a home-built electromagnet. All measurements were performed at room temperature.

Results

Fig. 2 shows measurements of the absorption changes at 820 nm (ΔA_{820}) in the μs and ns range. In accordance with Ref. 7, we attribute the decay in the ns range with $t_{1/2} \approx 20$ ns to charge recombination between P-700$^+$ and A_0^- (the somewhat longer halftime reported previously (30–50 ns) was probably due to the slower response of the apparatus). Magnetic fields up to 500 G did not significantly affect the half-time of this decay (changes of less than 10% would not have been detectable). However, a clear magnetic-field effect was observed in the μs range: the amplitude of the

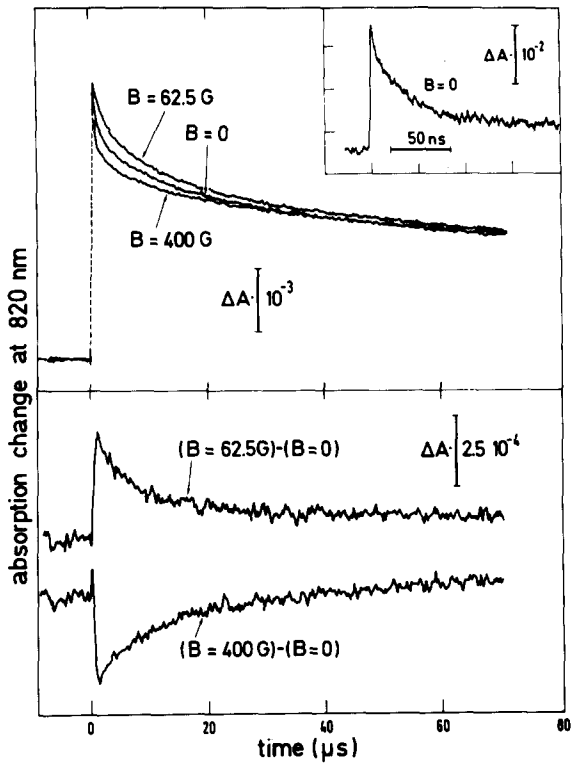

Fig. 2. Upper panel: absorption changes at 820 nm in the μs range for magnetic fields B = 0, 62.5 and 400 G and in the ns range for $B = 0$ (inset). Lower panel: differences between signals for $B = 62.5$ G and $B = 0$ (upper trace) and between $B = 400$ G and $B = 0$ (lower trace). Experimental conditions: CP1 particles in 50 mM Tris (pH 8.0), 2.0 μM dichlorophenolindophenol, 1 mM sodium ascorbate, 5 mM $Na_2S_2O_4$; absorbance in the red maximum at 675 nm = 20 (optical path: 10 mm); excitation: approx. 500 μJ/cm² per 30 ps pulse at 532 nm; average of 20 experiments, electrical bandwidth 500 Hz–500 MHz (ns range, inset) or 100 experiments, bandwidth DC–1 MHZ AC (μs range).

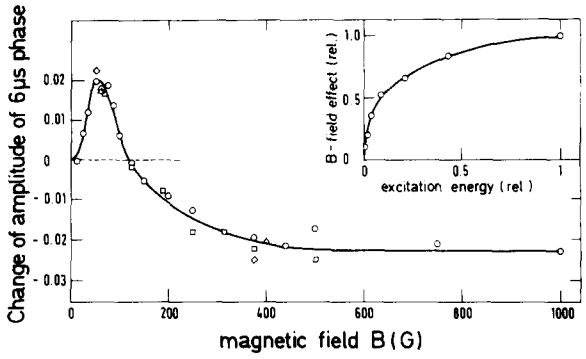

Fig. 3. Magnetic-field dependence of the change of amplitude of the 6 μs phase of ΔA_{820} relative to ΔA_{820} at 4 ns after excitation. Four series of measurements with different batches of CP1 particles are displayed by the different symbols. Experimental conditions as for Fig. 2, except that $Na_2S_2O_4$ was omitted in two series (circles and squares). Inset: magnetic field effect on the 6 μs phase as a function of excitation energy. Each point represents the change of amplitude of the 6 μs phase between a measurement at 400 G and a measurement at 62.5 G. A relative energy of 1 corresponds to approx. 5 mJ/cm² per 30 ps pulse at 532 nm. Other experimental conditions are as for Fig. 2.

$t_{1/2} \approx 6$ μs phase, which is attributed to the decay of ^{3}P-700 [8] is increased by weak fields (around 60 G) and decreased beyond approx. 120 G (Figs. 2 and 3) when compared to the zero-field signal. A slight decrease of this phase has already been observed for CP1 particles [8], but the range below 200 G had not been explored.

It should be mentioned that the signals depicted in Fig. 2 were measured in the presence of 5 mM $Na_2S_2O_4$ in order to diminish the concentration of O_2 in the sample. Omission of $Na_2S_2O_4$ did not significantly change the signal in the ns range and the magnetic-field effect represented by the difference between signals at $B \neq 0$ and $B = 0$ as those depicted in Fig. 2, lower pannel. However, a decay phase with $t_{1/2} \approx 300$ μs (in the presence of $Na_2S_2O_4$) was accelerated to a few microseconds in the absence of $Na_2S_2O_4$ (that is in the presence of O_2). We attribute this phase to the decay of the triplet state of some disconnected antenna chlorophyll molecules (possibly due to the presence of SDS), which is quenched by O_2 (the lifetime of ^{3}P-700 is, however, fairly independent of the presence of O_2 [8]).

The change of amplitude of the 6 μs phase depicted as a function of magnetic field in Fig. 3 has been plotted relatively to ΔA at 4 ns after excitation (taking a value of 1 for $\Delta A_{4\ ns}$), which represents approximately the initial absorption of ($P\text{-}700^+ - A_0^-$). In order to give an estimate of the change in yield of ^{3}P-700, we assume that the absorption of ($P\text{-}700^+ - A_0^-$) (with A_0 probably being chlorophyll *a* [9]) is roughly twice that of ^{3}P-700 at 820 nm. On these premises, the maximum around 60 G would represent an increase in yield of ^{3}P-700 by 4% (of all ($P\text{-}700^+ - A_0^-$) formed initially) and the effect at 1000 G a decrease by

4.5%, compared to $B = 0$. Assuming additionally that the 6 μs phase in the presence of $Na_2S_2O_4$ represents all ^{3}P-700 and only ^{3}P-700, the yield of formation of ^{3}P-700 from (P-700$^+$–A_0^-) would be in the order of 15% for $B = 0$. Obviously, this is a very rough estimation. Previous measurements using different experimental conditions (excitation, reaction medium) and a different approach, indicated that the yield of ^{3}P-700 in CP1 particles is in the order of 60% [7], while lower values of approx. 5% were also determined [10]. Experiments are in progress to resolve these discrepancies.

The inset of Fig. 3 shows the magnetic-field effect on the 6 μs phase as a function of excitation energy (a relative energy of 1 corresponds to approx. 5 mJ/cm^2 per pulse). This saturation curve is consistent with the saturation behavior of other reactions in Photosystems I and II for flash excitation at 532 nm [11–13] if one takes the different antenna sizes into account. This confirms that the observed magnetic field effect is related to reaction center photochemistry and not to, for instance, disconnected chlorophylls.

As a further proof for the assignment to the reaction center, we checked that the magnetic-field effect disappears if P-700 is chemically oxidized by ferricyanide (not shown). Under these conditions, the ΔA decay with $t_{1/2} \approx 20$ ns was suppressed, but there remained a signal with a decay halftime of approx. 2 μs and an initial amplitude of $\Delta A = 1.5 \cdot 10^{-3}$ (under conditions as in Fig. 2). We attribute this signal to the triplet state of disconnected antenna chlorophylls which is quenched by O_2. The absence of a magnetic-field effect on this signal (not shown) confirms that the field effect described above is related to the functional reaction center of photosystem I.

Discussion

The magnetic-field dependence of the yield of formation of ^{3}P-700 (Fig. 3) is strikingly different from the corresponding effect in bacterial reaction centers, where the yield of ^{3}P formation decreases continuously with magnetic field (for $B \leqslant 1000$ G) (see Ref. 1 for a review; a differing result was recently obtained from a reaction-yield-detected magnetic resonance experiment [14], which can be interpreted in the same way as an experiment on the magnetic-field modulation on the reaction yields [15]; see also Ref. 4).

However, such field-effect curves exhibiting a relative maximum were theoretically predicted for radical pairs with a sufficiently large (larger than hyperfine couplings or lifetime broadenings) magnetic interaction between the members of the pair [2,16]. When the field is increased, the $T_{\pm 1}$ states split away from the T_0 state and in that case, when one of the $T_{\pm 1}$ states (depending on the sign of the exchange interaction) crosses the S level, the singlet-triplet mixing is more allowed due to the degeneracy of the energies.

Another condition apparently to be met to get this kind of curve is also that one type of magnetic interaction (dipolar or exchange) predominates over the other one, and in case of an important dipolar interaction, this one should be axial or near-axial [2] (in the following, the exchange interaction will be denoted 2J, corresponding to an energy splitting $E_S - E_T = 2J$, and the dipolar interaction will be described by the zero-field splitting parameters D and E). Dipolar tensors are expected to be near-axial for radical pairs (i.e., $|D| \gg |E|$) and this has been calculated to be the case, on the basis of X-ray structure, for the dipolar interaction between P^+ and A_0^- in the reaction center of *Rhodopseudomonas viridis* (where A_0 is a bacteriopheophytin molecule) [17]: $|E| \ll 0.5$ G and $D = -11$ G. If a dipolar interaction is the major magnetic interaction betwen P-700$^+$ and A_0^-, its $|D|$ value should be larger than 120 G, if extrapolation of the curves in Ref. 2 is made for larger $|D|$ values. The comparison with bacterial reaction centers makes this hypothesis unlikely, but it cannot be excluded. Thus the exchange interaction is probably the major magnetic interaction between P-700$^+$ and A_0^-. The corresponding singlet-to-triplet energy splitting should be about 60 G to accommodate the data of Fig. 3. More precisely, we have to hypothesize that both interactions, with exchange being significantly larger than dipolar (so that the maximum of the curve is not damped out [2]), are to be taken into account for the pair (P-700$^+$–A_0^-). These conclusions confirm a previous estimation of the exchange interaction inside the primary radical pair [4]. In this study, a value comprised between

60 and 100 G ($|2J|$) was derived from the magnetic-field dependence of PS I fluorescence.

These conclusions raise several questions, particularly when they are compared to the data obtained on bacterial reaction centers:
- an exchange interaction $|2J|$ of 60 G appears much larger than the upper bound of 20 G found in bacterial reaction centers [3]. Such a large interaction may involve a superexchange process.
- a 15% yield of formation of ^{3}P-700 in zero-field may appear high with an exchange interaction of about 60 G, but this can be explained by a significantly smaller value of the decay rate constant k_S in Photosystem I compared to purple bacteria.
- $|D| < |2J|$ contrasts with a recent estimation of $|D|/|J| > 20$ made from a line-shape analysis of polarized EPR signals in Photosystem I [18]. The possibility cannot be excluded that this is due to the difference in biological materials, namely that the exchange interaction is much larger in SDS-treated CP1 particles, as this parameter is an exponential function of distance and may vary considerably with some small conformational changes.

Another difference between our results on Photosystem I and the results for bacterial reaction centers (where a low magnetic field decreases by 20–40% the decay rate constant of the radical pair [19,20]) is that the recombination kinetics of the pair ($P\text{-}700^+\text{–}A_0^-$) was not markedly changed by the magnetic fields applied in this study. Two remarks can be made concerning this discrepancy.
(i) As pointed out in Ref. 19, the radical-pair mechanism, as outlined above, predicts that the ratio of the decay rate constants in the presence $k(B)$ and in the absence $k(0)$ of magnetic field should be:

$$\frac{k(B)}{k(0)} = \frac{1-(1-k_S/k_T)\Phi(0)}{1-(1-k_S/k_T)\Phi(B)}$$

(expression derived from Refs. 21 and 22), where $\Phi(0)$ and $\Phi(B)$ are the triplet yields in the absence and presence of a magnetic field, respectively. When the ratio k_S/k_T is varying from zero to infinity, the ratio $k(B)/k(0)$ should therefore increase continuously from $(1-\Phi(0))/(1-\Phi(B))$ to $\Phi(0)/\Phi(B)$ when $\Phi(0) > \Phi(B)$, or decrease continuously between the same values when $\Phi(0) < \Phi(B)$. From this dependence and taking into account the preceding yields of formation of ^{3}P-700 ($\Phi(0\text{ G}) = 0.15$; $\Phi(60\text{ G}) = 0.19$; $\Phi(1000\text{ G}) = 0.105$), it can be shown from simple calculations that an upper bound of 10% for the magnetic field effect on k (i.e., $0.9 < k(B)/k(0) < 1.1$ for any field B) corresponds to an upper bound of 4 for the ratio k_S/k_T. Therefore, taking into account the signal-to-noise ratio of our nanosecond experiments, a magnetic field effect on k would have been hardly detectable for $k_S/k_T < 4$.
(ii) The observed decay of ΔA_{820} with $t_{1/2} \approx 20$ ns in CP1 particles represents the superposition of the charge recombination kinetics in the pair ($P\text{-}700^+\text{–}A_0^+$) and of the rise kinetics for the formation of ^{3}P-700. A magnetic field effect on the decay rate of the pair might be partly obscured by an effect on the formation rate of ^{3}P-700.

Hence there is no obvious contradiction between the clearly resolved magnetic-field effect on the yield of ^{3}P-700 (Fig. 3) and our observation that concomitant changes in the halftime of ΔA_{820} in the ns range are surely below 10%.

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