DETECTION OF TRIPLET STATES IN ALGAE BY ZERO-FIELD RESONANCE

Sievert J. van der Bent,* Tjeerd J. Schaafsma,* and Joop C. Goedheer**

*Laboratory of Molecular Physics, Agricultural University,
De Dreijen 6, Wageningen, The Netherlands and
**Biophysical Research Group, Physics Institute,
The State University, Utrecht, The Netherlands

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SUMMARY Triplet states have been detected and characterized by zero-field splitting parameters in Anacystis nidulans, Euglena gracilis, Porphyridium cruentum, and Chlorella vulgaris, using fluorescence-detected magnetic resonance in zero-field at 4.2 K. Monitoring the 720 nm photosystem I emission, transitions between triplet spin levels have been assigned to antenna chlorophyll of one of both photosystems; photochemical reactions of chlorophyll are observed in the presence of an inhibitor and strong light, probably resulting in photoreduction and pheophytinization.

INTRODUCTION Triplet states have been detected in several photosynthesizing organisms (1-4) using magnetic resonance methods. The recently developed method of FDMR (5,6) appears to be very suitable to study the primary processes in photosynthesis using the triplet state as an internal probe, by monitoring fluorescence bands assigned to different parts of the PSU. In contrast with singlet states, triplet states are perturbed by (short-range) magnetic- rather than electric fields, and thus reflect molecular properties of pigments in the PSU, more than environmental effects. Here we report FDMR experiments on four types of algae, (blue-green) Anacystis nidulans, (red) Porphyridium cruentum and (green) Chlorella vulgaris and Euglena gracilis. No FDMR signals were obtained for Synechococus cedrorum, Phaeodactylum tricornutum and Visscheria stellata.

METHODS Immediately before experiment, algae were suspended in ethyleneglycol/H_2O 2:1 mixture, forming a glass at 4.2 K by immersion into liquid helium. Blue light (400-525 nm) excites the sample by reflection from a

Abbreviations:
FDMR: fluorescence detected magnetic resonance in zero magnetic field.
PSU: photosynthetic unit

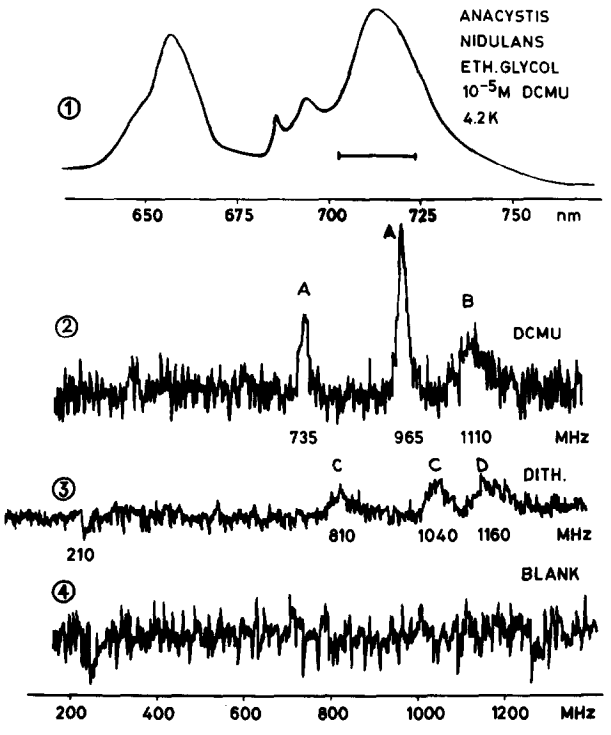

Figure 1 Surface-detected fluorescence spectrum of Anacystis nidulans in ethylene-glycol/H_2O 2:1 glass at 4.2 K in the presence of 10^{-5} M DCMU.

Figure 2 FDMR spectrum of Anacystis nidulans detected at 713 nm Chl fluorescence in the presence of 10^{-5} M DCMU. Detection bandwidth 40 nm.

Figure 3 FDMR spectrum of same alga with DCMU replaced by 10^{-3} M dithionite; other experimental conditions identical to those of Fig. 1,2.

Figure 4 Blank experiment of alga without blocking agent; other experimental conditions identical to those in Figs. 1-3.

dichroic mirror, transmitting fluorescence (> 600 nm) to an RCA 31034 photomultiplier cooled to -35°C, after passing through a .25 m Spex Minimate monochromator. Variable frequency microwave radiation was applied employing a helical slow-wave structure, surrounding the alga sample Fluorescence was monitored when the microwave frequency was swept through the 10-1300 MHz range. For further details, we refer to ref. 7.

RESULTS AND DISCUSSION If the main Chl- fluorescence band (see Fig. 1) of Anacystis nidulans at its maximum at 713.5 nm (with a 20 nm bandwidth) is monitored with microwaves swept from 10-1300 MHz, FDMR spectra were measured

as shown in Figs. 2, 3 if DCMU (10^{-5} M) or dithionite (10^{-3} M) was added before illumination. In the absence of inhibitors, no spectrum was observed (Fig. 4). Table I collects triplet state parameters of Anacyst and three other algae, in additon to those of some relevant compounds in vitro.

With Anacystis, four different types of FDMR spectra can be distinguished. In the presence of DCMU, and monitoring fluorescence at $\lambda_F \approx 713.5 \pm 20$ nm, transitions A and B are detected, as shown in Fig. 2; at $\lambda_F = 738 \pm 20$ nm, only A transitions are observed, whereas no resonances are found at $\lambda_F \approx 660$ nm (phycocyanineband) and $\lambda_F = 685$ and 694 nm (bands of P.S. II antenna Chlorophyll (8)). Thus, A and B transitions have a separate origin. It was concluded that resonances at 965 and 735 MHz correspond to D+E and D-E transitions of one and the same species A, whereas the broad transition at 1110 MHz is D+E of a different species B, the D-E transition of which is obscured by the D+E resonance of species A (9). Repeating the experiment with dithionite instead of DCMU eventually leads to disappearance of A and appearance of a similar, but displaced FDMR spectrum, with components C and D (Fig. 3). This may suggest that formation of species C occurs via the triplet state of A. Although the Chl 713 nm fluorescence decreases to roughly half of its original value during irradiation for $\sim$8 hrs, the species A resonances are completely replaced by B; this means that only those Chl molecules which are observable as triplets, are completely photo-transformed into species B. The latter one is tentatively assigned as photoreduced Chl.

With Chlorella in strong blue light illumination, FDMR spectra of type A were observed even in the absence of DCMU. Prolonged ($\sim$8 hrs) illumination with blue light at 4.2 K and in the presence of DCMU, resulted in a super-position of type A and C transitions, whereas B and D resonances are absent when measured close to the 720 fluorescence maximum. With Euglena (+DCMU),

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Chl = chlorophyll-a; DCMU = 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; P.S. = photosystem.

Table I. ZFS parameters of algae triple states

Alga[a]		$\|D\| \times 10^4$ cm^{-1}[b]	$\|E\| \times 10^4$ cm^{-1}[b]
Anacystis nidulans			
	A	283	38
	B	348	21
	C	311	38
	D	366	20
Porphyridium cruentum		283	37
Euglena gracilis		297	37
Chlorella	A	288	38
	C	311	38
Chlorophyll-a in EtOH		287	36
Pheophytin-a in MTHF[c]		341	33

a. Measured at T = 4.2 K; b. ZFS parameters defined as $|D| = \frac{3}{2}|Z|$, $E = 1/2|X - Y|$, where X, Y, Z define energies of triplet spin states in zero magnetic field; c. MTHF = 3-methyltetrahydrofuran.

type A resonances were detected only in the early stages of the experiment, the final result being of the C type, but without D.

By comparison with in vitro ZFS values (Table I), the transitions A found in Anacystis are assigned to monomeric Chl, being part of the P.S. I or II antenna pigment, since reaction center Chl is expected to have smaller ZFS parameters due to spin delocalization in a special Chl pair or oligomer, analogous to bacterial reaction centers (1,2). Since no resonances are observed at 685 and 695 nm, known to be associated with P.S. II antenna

ZFS = zero field splitting

chlorophyll, whereas on the other hand these bands are enhanced by DCMU (10), further experiments should decide in which part of the PSU species A is located. Species B may be pheophytin-a, resulting from known photoconversion of Chl (11), in view of its in vitro ZFS parameters (Table I). In the presence of a strong reductant, such as dithionite, triplet state Chl (species A) may be photoreduced to a compound with unknown structure, emitting fluorescence at 620, 651 and 731 nm (12), and thus observable as species C in the monitoring fluorescence band (700 ± 40 nm in this experiment). If C is photoreduced Chl, it can be readily pheophytinized to D (12). Further work on isolated chlorophyll-protein complexes is in progress.

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